JOURNAL
OF THE
FISHERIES RESEARCH BOARD
OF CANADA

JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

The Journal of the Fisheries Research Board of Canada is devoted to original research papers and other contributions dealing with matters of interest to Canada's fisheries and aquatic sciences. Papers submitted must be clearly written and must make a definite contribution to knowledge. Papers may be published in English or French, with abstracts in both languages.

Contributors' guidelines and notices are given in each issue of the Journal.

The Journal, or any of its volumes of its predecessors, has been published continuously since 1901 as: Contributions to Canadian Biology, 1901-1921; Contributions to Canadian Biology and Fisheries, New Series, 1921-1936; Journal of the Biological Board of Canada, 1937-1968.

The Journal is published in several volumes of twelve numbers. The number (Vol. 27, No. 12) was issued on February 10, 1970.

Editor:

J. C. Stevenson, Ph.D.

Associate Editor: L. W. Billingsley, Ph.D.
Associate Editor: R. H. Wiswar, M.Sc.

Production: M. L. MacIntyre, Marilyn Cammidy, B.A.

Fisheries Research Board of Canada
Office of the Editor
116 Lisgar Street
Ottawa 4, Ont., Canada

Manuscripts for publication should be addressed to the Editor

---

All Fisheries Research Board of Canada publications are for sale by the Queen's Printer, Ottawa. Remittances must be in advance; payable in Canadian funds to the order of the Receiver General of Canada. Subscription rates to the Journal are Canada, British Guiana, Hawaii, $8.00; single issue $1.00; to other countries $9.75 and $1.25.

The Fisheries Research Board of Canada also publishes a Bulletin series (brief articles), an Annual Report, and a biennial series of investigations. All publications may be purchased at Book sales agencies located in Ottawa, Toronto and Vancouver, B.C.; in Winnipeg, Man.; the Maritime Provinces and Quebec Province, Que.; St. John's, Nfld.; Halifax and Dartmouth, N.S.; Moncton, N.B.; and St. John's, Nfld.

ANNOUNCEMENT

Because of increasing publishing costs a charge of $20.00 per printed page is made for manuscripts published in the *Journal of the Fisheries Research Board of Canada*. Manuscripts received after *October 31, 1969*, are subject to this charge. Authors should submit a letter stating the willingness of their institution to accept the charge.

AVIS

Etant donné l'augmentation des frais de publication, le tarif de publication est de $20 la page imprimée pour les manuscrits publiés dans le *Journal of the Fisheries Research Board of Canada*. Les manuscrits reçus après le *31 octobre 1969* sont assujettis à ce tarif. L'auteur devra présenter une lettre où il indiquera que son employeur accepte de se charger de cette dépense.

An Analysis of Variation of Insecticide Residues in Landlocked Atlantic Salmon (*Salmo salar*)[1]

RICHARD B. ANDERSON[2] AND OWEN C. FENDERSON

Maine Department of Inland Fisheries and Game
Augusta, Maine 04330, USA

ANDERSON, R. B., AND O. C. FENDERSON. 1970. An analysis of variation of insecticide residues in landlocked Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 27: 1–11.

High variation in yearly samples of insecticide residues found in landlocked salmon from Sebago Lake, Maine, has necessitated an evaluation of sampling methods and an investigation of causes of variation. A sample of 59 male salmon, composed of ages III+, IV+, and V+, and representing fish in poor and good body condition was collected from a spawning run in the fall of 1967 and analyzed for DDT, DDD, DDE, dieldrin, and fat content. Levels of insecticide residues were found to be highly dependent on age and fat content. DDD and DDE increased with age and fat content, and dieldrin increased with fat content. Age and fat content were interdependent in their effects on DDT levels. DDT was significantly higher in high-fat than in low-fat fish at ages III+ and IV+ but not at age V+, showing a decrease with age among fish with high fat content and remaining constant with age among fish with low fat content. It is recommended that completely random selection of fish for insecticide analyses be abandoned in favor of stratification by sex, age, and fatness. Some of the possible causes of variation in insecticide content of salmon are discussed.

Received July 25, 1969

INTRODUCTION

TO PROPERLY EVALUATE insecticide concentrations in fish and make comparisons between fish collected from different waters or within the same body of water in different years, it is necessary to determine the degree of confidence that can be placed on the accuracy of samples. Several authors have noted that DDT levels in fish tend to be highly variable and have implicated certain factors, particularly body fat, as influencing accumulation of DDT. Kleinert et al. (1968) reported that data collected on the DDT content of Wisconsin fish suggested a positive relationship between fat percentages and DDT levels in fish, but that the relationship was not simple and direct, and that other factors might be involved. Burdick et al. (1964) reported that yearly differences in DDT content of individual lake trout were evident, but they found that individual determinations were too variable and numbers of fish too few to indicate any trend in DDT concentrations. Holden (1966) stated that the effects of pesticide residues on fish, as on other vertebrates, depend in part on the

[1]This research was supported in part by Federal Aid, Dingell-Johnson Act (F-17-R, Maine).

[2]Present address: Portland Museum of Natural History, 22 Elm Street, Portland, Maine 04111.

Printed in Canada (J1542)

condition of the fish, which he believes is primarily determined by the lipid content of tissues.

Salmon from Sebago Lake, Maine, have been sampled annually since 1962 to determine the extent of insecticide contamination, and to determine if there was a trend for an increase or decrease of insecticide residues in the salmon population. High variation within and between yearly samples has precluded strict statistical interpretation of these data. The high cost of laboratory analyses and the expenditure of effort required to obtain random samples large enough to make meaningful estimates of insecticide levels in fish populations have necessitated a critical evaluation of sampling procedures.

In 1967, we selected 59 landlocked Atlantic salmon from Sebago Lake on the basis of sex, age, and body condition in an attempt to identify causes of variation in insecticide residues, and to establish criteria for selection of future samples. The results of an analysis of variables affecting levels of DDT, DDD, DDE, and dieldrin in these fish are the subject of this report.

MATERIALS AND METHODS

Landlocked salmon were collected by seining from the Jordan River, one of the major salmon spawning tributaries to Sebago Lake. All fish were of hatchery origin and of known age, as determined from inspection of finclips, and they had been reared to age I+ before they were stocked in Sebago Lake. Three age classes were represented in the samples. Ages III+, IV+ and V+ averaged 41.1, 43.9, and 48.0 cm in total length, and 527, 621, and 834 g in weight, respectively.

To eliminate sex-dependent variation and reduce variation due to spawning condition, only male salmon were selected for analyses. Results of past analyses indicated that large amounts of DDT, in proportion to total body weight, were accumulated in ovaries and the inclusion of females in various spawning stages would contribute to high sampling variation. Gonads of male salmon accumulate proportionally smaller amounts of DDT, and elimination of milt before capture would have little effect on the total body concentration of DDT.

Whole fish or parts of fish were grouped in the following manner for initial analyses.:

Group A — For this sample, 30 mature males were divided into three equal groups representing ages III+, IV+, and V+. Each age-group was subdivided into two equal groups of fish in poor condition and fish in good condition. The fish were chosen subjectively by inspection.

Group B — This sample consisted of 29 mature males (10 age III+, 10 age IV+, and 9 age V+) that were treated in exactly the same manner as those in group A, except that the brains were removed before the fish were analyzed.

Group C — This sample consisted of the brains from fish in group B. Each brain was numbered so that it could be identified with the fish in Group B from which it had been removed. Samples of brain tissue were analyzed separately on the basis of a report by Stickel et al. (1966) indicating that brain tissues of birds were less variable in DDT concentrations than other body tissues. It was felt that these findings might also apply to fish.

Groups A and B were later combined into one sample for reasons explained below.

Immediately after fish were killed and processed, the samples were placed in airtight polyethylene bags. They were then quick-frozen at −35 F within 6 hr after collection and stored at 0 F until analyzed. All assays were done by the Wisconsin Alumni Research Foundation, Madison, Wisconsin, using an electron capture gas chromatographic procedure described by the U.S. Department of Health, Education, and Welfare (1965).

Fat content, moisture, and insecticide residues are expressed as percentages of total wet weight. Condition factors of fish in groups A and B were computed by the standard formula:

$$K = 100{,}000\ W/L^3,$$

where K = condition factor, W = weight of fish, and L = length of fish.

RESULTS AND DISCUSSION

DDT and its analogs were present in the tissues of salmon 3 years after the cessation of spraying around the shores of Sebago Lake. Dieldrin was present in all fish, but endrin was detected in measurable amounts in only three fish. Endrin is not considered in this report.

Preliminary analyses of the data indicated that the brain samples would yield little, if any, useful information not obtainable from the other sample groups (Table 1). Insecticide levels were appreciably lower and more variable, and the fat content was higher in the brain samples than in the fish with the brains removed (group B) or in the whole fish (group A). Also, dieldrin was not detected in brain tissue and the brain samples were therefore excluded from the analyses. Comparisons of mean values, ranges, and standard errors of the variables measured for groups A and B justified combining these two

TABLE 1. Descriptions of samples from Sebago Lake salmon used in analyses of insecticide residues.

Sample group	Sample size	Variable	Mean	Range	SE of mean
Group A (whole fish)	30	Body length (*mm*)	444	371–538	7.74
		Body weight (*g*)	652	368–1360	42.35
		Condition factor	0.717	.564–.872	0.0159
		% moisture	77.0	71.3–84.4	0.53
		% fat	3.72	1.22–10.5	0.36
		DDE (*ppm*)	9.38	4.30–14.6	0.60
		DDD (*ppm*)	1.23	0.25–2.72	0.100
		DDT (*ppm*)	0.80	0.25–3.61	0.127
		Dieldrin (*ppm*)	0.019	0.011–0.037	0.001
Group B (brains removed)	29	Body length (*mm*)	444	378–531	7.77
		Body weight (*g*)	655	397–1133	36.97
		Condition factor	0.731	0.599–0.855	0.0125*
		% moisture	76.1	71.1–80.4	0.40
		% fat	3.77	1.38–8.59	0.31
		DDE (*ppm*)	8.61	4.47–15.4	0.57
		DDD (*ppm*)	1.00	0.30–2.52	0.086
		DDT (*ppm*)	1.36	0.30–3.18	0.134
		Dieldrin (*ppm*)	0.021	0.012–0.042	0.001
Group C (brains of group B)	29	% moisture	80.1	73.8–97.4	0.73
		% fat	8.99	1.11–12.86	0.40
		DDE (*ppm*)	2.54	0.49–9.00	0.43
		DDD (*ppm*)	0.20	0.05–0.64	0.02
		DDT (*ppm*)	0.72	0.18–3.67	0.115
		Dieldrin (*ppm*)	0.00	–	–

sample groups to increase the sample size to 59. Since the individual weights of brains and bodies of each fish were known, a few computations were done to adjust the values of mean residue for fish in group B to account for the loss of brains. This was accomplished by computing total parts of residue contained in the brain and body separately, adding the resultant values, and re-calculating parts per million residue for the whole body. However, brain weights comprised less than 0.1% of total body weight, and it was apparent that conversions would not change the values of mean residue unless an accuracy of more than three decimal places was desired.

Initial attempts to analyze the combined sample of 59 fish for possible relationships between age and fat content of salmon and insecticide concentrations by regression and one-way analysis of variance were discouraging. Conflicting results and extremely high and unequal variances indicated that larger samples were needed. Much more consistent and revealing results were obtained by grouping the samples by age and degree of fatness, and using a randomized, two-factor analysis of variance with interaction of main effects and Duncan's test for treating multiple means. Guilford (1965) defines interaction variance as those variations not attributable to either of two influences acting alone but to joint effects of the two acting together.

Three separate statistical analyses were performed using (1) age and percentage fat; (2) age and condition factor; and (3) age and visual condition as factors affecting variations in levels of DDE, DDD, DDT, and dieldrin in salmon. Fish were grouped into two categories on the basis of their fat content. Low-fat fish had $\leqslant 3.53\%$ fat and high-fat fish had $>3.53\%$ fat. Fish in poor condition had a condition factor of $\leqslant 0.723$ and fish in good condition had a factor of >0.723. The analysis with age and percentage fat was considered to be most important but the other analyses were done to determine whether salmon could be selected subjectively for fat content on the basis of visual inspection or by condition factor.

Except for some differences in the degree of statistically significant relationships, the three analyses yielded similar results (Table 2). The tests with age and visual condition as factors paralleled most closely those using age and percentage fat. Apparently, the grouping on the basis of visual inspection was as accurate or even more accurate than measuring condition factors.

The results of analysis of variance and Duncan's test revealed that age and fat content contributed significantly to the variation in insecticide residues (Table 3 and Fig. 1).

The amounts of DDD and dieldrin were significantly higher in fish with high fat content than in fish with low fat content (Fig. 1). DDE also tended to be higher in high-fat fish but the relationship was not significant ($p > 0.05$). DDE and DDD levels were higher in older fish than in younger fish. Dieldrin levels did not vary consistently with age. A significant interaction for DDT revealed that the main effects of age and fat were not independent in their influence on levels of DDT (Table 3). The F′ test indicated that neither factor was significant when considered alone but that DDT varied under the combined

TABLE 2. Summary of analysis of variance of DDE, DDD, DDT, and dieldrin in salmon from Sebago Lake.

Measurement	Factors		
	Age and fat	Age and condition factor	Age and visual condition
DDE (*ppm*)	Trend to increase with fat NS[a] Increases with age[b]	Does not vary with condition factor NS Increases with age[b]	Does not vary with visual condition NS Increases with age[b]
DDD (*ppm*)	Increases with fat[b] Increases with age[b]	Trend to increase with condition factor NS Increases with age[b]	Increases with visual condition[c] Increases with age[b]
DDT (*ppm*)	Main effects not significant alone but show significant *interaction*: DDT varies under combined effects of age and fat[c]	Trend for interaction of main effects NS	Main effects not significant alone but show significant *interaction*: DDT varies under combined effects of age and visual condition[c]
Dieldrin (*ppm*)	Increases with fat[b] Does not vary with age NS	Trend to increase with condition factor NS Trend to increase with age NS	Increases with visual condition[c] Trend to increase with age NS

[a]No statistically significant relationship.
[b]Significant F-ratio at 0.01 level.
[c]Significant F-ratio at 0.05 level.

influence of fat and age. At age III+ and IV+, DDT levels were significantly higher in fish with high fat content than in fish with low fat content, but this relationship was not evident for age V+ fish (Fig. 1). The interaction effect was caused by a pronounced tendency for DDT to decrease with increasing age in fish with a high fat content, whereas DDT levels remained constant with age in fish with low fat content.

The interaction of fat and age had a marked influence on the results of initial attempts to analyze the relationship between DDT and percentage body fat by regression analysis. With the combined sample, a significant linear regression was obtained for the relationship between fat and DDT ($F = 42.44$, df $= 1, 58$, $p < 0.01$). However, a low r^2 of 0.43 indicated that the relationship was not a strong one. Therefore, a separate regression analysis was done for each age-group (Fig. 2). A strong relationship between percentage fat and DDT was shown for age III+ fish, and a still significant but weaker relationship was obtained for age IV+ fish. For age V+ fish, there was no significant relationship between fat and DDT. Logarithmic transformations applied to the data did not appreciably improve goodness of fit of the regression lines.

TABLE 3. Results of analysis of variance test for variation in amounts of DDE, DDD, DDT, and dieldrin with percentage fat and age of Sebago Lake salmon.

Variable	Source of variation	df	Sums of squares	Mean squares	F	F′
DDE	Fat	1	15.883	15.883	2.81	
(*ppm*)	Age	2	253.334	126.668	22.38[a]	
	Fat × age	2	11.130	5.565	0.98	
	Error	53	299.999	5.660		
	Total	58	591.453			
DDD	Fat	1	1.703	1.703	8.20[a]	
(*ppm*)	Age	2	2.468	1.234	5.95[b]	
	Fat × age	2	0.293	0.147	0.71	
	Error	53	11.003	0.208		
	Total	58	15.512			
DDT	Fat	1	8.438	8.438		5.02
(*ppm*)	Age	2	1.920	0.960		0.57
	Fat × age	2	3.360	1.680	4.56[b]	
	Error	53	19.511	0.368		
	Total	58	33.462			
Dieldrin	Fat	1	0.4123×10^{2}	0.4123×10^{3}	9.70[a]	
(*ppm*)	Age	2	0.1549×10^{3}	0.7746×10^{4}	1.32	
	Fat × age	2	0.8166×10^{5}	0.4083×10^{5}	0.10	
	Error	53	0.2254×10^{2}	0.4252×10^{4}		
	Total	58	0.2775×10^{2}			

[a]Significant at the 0.01 level
[b]Significant at the 0.05 level.

DDT levels in salmon collected from the Jordan River spawning run from 1962 through 1967 have been highly variable (Table 4). Analysis of samples from 1962 through 1965 were reported previously (Anderson and Everhart, 1966). Until 1967, samples were selected to duplicate the first year's sample that consisted of 10 randomly selected salmon of each sex of each age class from II+ through VI+, and fish in poor body condition and good body condition. A decrease in DDT levels from the highest level in 1965 is consistent with the fact that spraying operations around the shores of Sebago Lake were discontinued in 1964. However, strict statistical interpretation of trends in DDT contamination of salmon has been negated by wide confidence limits about sample estimates in some years, and a pronounced tendency for variances to increase as means increase. The cause of the latter phenomenon is not known, but may be related to the increased chance for DDT contamination to vary among individual salmon as they are exposed to a wider range of insecticide levels.

If a standard error of 10% of the mean is acceptable, then only the 1967 combined sample and subsample of age III+ fish with a high fat content would

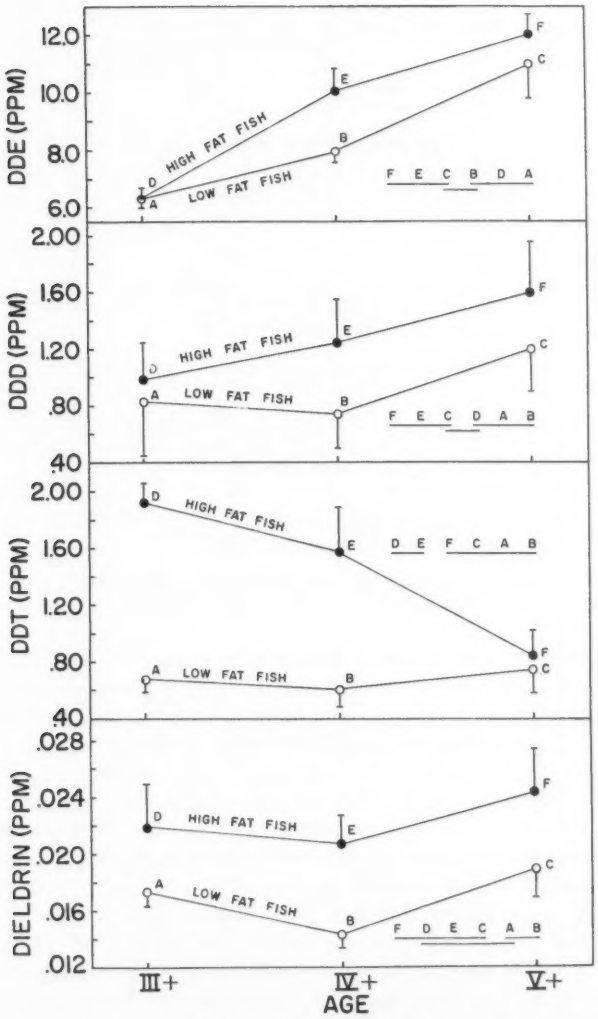

FIG. 1. Amounts of DDE, DDD, DDT, and dieldrin in salmon with low and high fat content at age III+, IV+, and V+. Vertical lines attached to mean points represent one standard error from the mean. Letters identifying means and underlined by a common line are not significantly different and those not underlined by a common line are significantly different (Duncan's test at the 0.05 level). Note that the increment scale for ppm varies; comparative differences, therefore, are not all of the same magnitude.

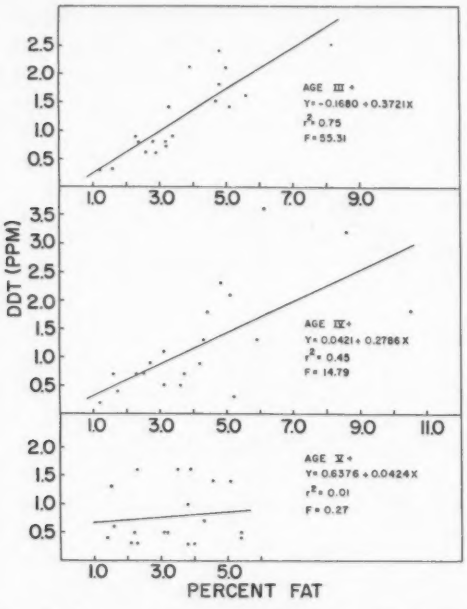

FIG. 2. Linear regressions of DDT on percentage body fat of salmon at age III+, IV+, and V+.

meet the criterion. The required sample size needed to predict mean DDT levels with an accuracy of 10% is prohibitively high in all sample groups, except age III+ fish with a high fat content (Table 4). Judging from the variation in the larger combined sample of 1967, which included only males, it might be unrealistic to expect that varitaion could be reduced any further in sampling mean levels of DDT contamination in the salmon population. Insecticide contamination is probably inherently variable, and disproportionate means and variances might be expected among age-groups that have been exposed to insecticides to varying degrees or for varying periods of time and among individuals having different physiological attributes.

In spite of the large variances among the small 1967 subsamples, the differences in DDT residues in fish of different ages and fat content are probably too great to be attributable to chance alone. There was no overlap, for example, in the confidence limits about mean residues for low- and high-fat fish at ages III+ and IV+ (Table 4). The analysis indicates that a sample favoring young, low-fat salmon taken in 1 year and compared with another year's sample favoring high-fat fish of the same age would lead to erroneous conclusions concerning true differences between years. Significant differences might only reflect physiological differences between fish in the two samples, rather than true differences in the mean level of contamination in the population between years. Furthermore, a single sample favoring high-fat fish in any one

TABLE 4. Variation in DDT levels in yearly samples of Sebago Lake salmon collected from 1962 to 1967.

Year	Sample characteristics	N	Mean (*ppm*)	Variance	SE	Confidence limits	Estimated sample size[a]
1962	All fish combined	10	1.10	0.318	0.178	0.70–1.50	134
1963	" " "	10	3.20	8.038	0.897	1.20–5.20	402
1964	" " "	10	1.76	1.631	0.404	0.86–2.66	270
1965	" " "	10	6.92	15.143	1.231	4.87–9.42	162
1966	" " "	10	2.74	6.713	0.819	0.89–4.59	457
1967	" " "	59	1.07	0.577	0.099	0.87–1.27	202
1967	Age III+, low fat	12	0.68	0.104	0.093	0.48–0.88	110
1967	Age III+, high fat	8	1.92	0.162	0.142	1.58–2.26	25
1967	Age IV+, low fat	7	0.60	0.810	0.106	0.34–0.86	143
1967	Age IV+, high fat	13	1.81	1.370	0.324	1.10–2.52	202
1967	Age V+, low fat	10	0.75	0.268	0.163	0.38–1.12	245
1967	Age V+, high fat	9	0.85	0.260	0.170	0.46–1.24	190

[a]Estimates of sample size needed, based on sample variances, to predict mean ppm DDT within 10% at the 95% level of confidence were computed from the formula: $n = (t^2)\ (s^2)/E^2$, where n = estimated sample size, t = table value for $n_1 - 1$ degrees of freedom, s^2 = sample variance, and E = specified error (Freese, 1967).

year would place the mean level of DDT contamination of the population too high; conversely, a sample favoring low-fat fish would place the level of contamination too low.

The data presented in this report demonstrate that caution is required in the selection of landlocked salmon, and probably other fishes as well, for the purpose of evaluating levels of contamination by insecticides in aquatic environments. The common practice of selecting small samples of fish completely at random for monitoring insecticides, without regard to factors causing variation, may result in biased comparisons reported to show true increases or decreases in levels of contamination. It is recommended that the completely random method of selection of fish for insecticide analysis be abandoned in favor of a stratified random method in which sample fish would be chosen for common attributes of origin, age, sex, fatness, and possibly other factors contributing to variation in insecticide levels.

We can only speculate on the causes for the variation of insecticide residues in the tissues of Sebago Lake salmon revealed by this study. The high residue levels in fish with high fat content compared with those in fish with low fat reserves is expected because fat fish have a greater capacity to store fat-soluble hydrocarbons. The accumulation of residues should increase with age, because older salmon would have been exposed longer to persistent insecticides. The interaction between the effects of age and fat content and their combined influence on DDT levels is less easily explained. Possibly the decrease of DDT with age in high-fat fish and constancy of low levels of DDT in low-fat fish may reflect differences in mortality among age-groups, differences in feeding rates and assimilation of insecticides by fish in poor and good condition, or differences in metabolic rates of fish of different ages and fat content. For example, the older fish in our samples may be individuals from the population that had survived low, sublethal doses of DDT, whereas fish that had received high, lethal doses would have died and not been included in our samples. The apparent decrease of DDT with age in high-fat fish may then only represent sampling anomalies rather than physiological differences in metabolism of DDT by fish with low and high fat reserves. However, true physiological differences between fish of different degrees of fatness may indeed complicate age-dependent relationships. Fat, 3-year-old salmon, for example, are probably the fastest growing individuals in the population, and may have the greatest capacity to assimilate and store DDT. DDT levels may be less dependent on age in fish with low fat reserves that have a low capacity to store fat-soluble hydrocarbons and have comparatively high rates of catabolic breakdown and excretion of DDT. Also, salmon in poor condition may assimilate less DDT from their environment because of a reduced capacity to take food.

ACKNOWLEDGMENTS

We wish to express our gratitude to Mr Stuart DeRoche, Mr Robert Rupp, and Dr Harry Everhart for their assistance and advice. Appreciation is also extended to Mr Lyndon Bond, Mr Donald Mairs, Dr Richard Hatch, and Dr John Dimond for their critical reviews of the manuscript.

REFERENCES

ANDERSON, R. B., AND W. H. EVERHART. 1966. Concentrations of DDT in landlocked salmon (*Salmo salar*) at Sebago Lake, Maine. Trans Amer. Fish. Soc. 95: 160–164.

BURDICK, G. E., E. J. HARRIS, H. J. DEAN, T. M. WALKER, J. SKEA, AND D. COLBY. 1964. The accumulation of DDT in lake trout and the effect on reproduction. Trans. Amer. Fish. Soc. 93: 127–136.

FREESE, F. 1967. Elementary statistical methods for foresters. U.S. Dept. Agr., Agr. Handb. 317: 87 p.

GUILFORD, J. P. 1965. Fundamental statistics in psychology and education. McGraw-Hill Book Co., New York. 605 p.

HOLDEN, A. V. 1966. Organochlorine insecticide residues in salmonid fish. *In* N. W. Moore [ed.] Pesticides in the environment and their effects on wildlife. J. Appl. Ecol. 3(Suppl.): 45–53.

KLEINERT, S. J., P. E. DEGURSE, AND T. L. WIRTH. 1968. Occurrence and significance of DDT and dieldrin residues in Wisconsin fish. Wis. Dep. Natur. Resources, Technol. Bull. 41: 43 p.

STICKEL, L. F., W. H. STICKEL, AND R. CHRISTENSEN. 1966. Residues of DDT in brains and bodies of birds that died on dosage and in survivors. Science 151: 1549–1551.

UNITED STATES DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE. 1965. Pesticide analytical manual, Vol. I. Food and Drug Admin., Washington, D.C., Rev. 1965.

Grazing Rates and Food Selection in *Diaptomus oregonensis* (Copepoda) from Marion Lake, British Columbia[1]

DONALD J. MCQUEEN

Institute of Animal Resource Ecology
University of British Columbia, Vancouver, B.C.

MCQUEEN, D. J. 1970. Grazing rates and food selection in *Diaptomus oregonensis* (Copepoda) from Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 27: 13–20.

The filtration and ingestion rates of *Diaptomus oregonensis* females and stage V copepodids were examined in three experimental situations. When unialgal cultures of 20,000 cells per milliliter were offered to *D. oregonensis* the filtration rates remained constant for cells ranging from 120 to 10,000 μ^3. When a mixture of diatoms was offered the animals did not filter cells ranging from 1 to 60 μ^3 despite the fact that the cells in this size range were concentrated at about 20,000 per milliliter. When natural phytoplankton from Marion Lake was used as a food source, the filtration rates were low for cells smaller than 100 μ^3, increased to a maximum of 12.9 ml per animal per day for cells ranging from 102 to 333 μ^3, and remained constant with increased cell volume and decreased cell concentration. Several species of *Cyclotella* and *Merismopedia* were not removed from the Marion Lake plankton by *D. oregonensis*.

Received July 28, 1969

INTRODUCTION

THE MAJOR FACTORS that influence the ability of filter-feeding copepods to filter and ingest phytoplankton might be divided into three broad categories. Filtration rates are determined by animal size, by climatic factors such as light and temperature (Fuller, 1937; Conover, 1956), and by phytoplankton size, morphology, and concentration. Cell concentration is, in a general way, directly related to ingestion rates and inversely related to filtration rates (Marshall and Orr, 1955; Mullin, 1963; Richman, 1966). Parsons et al. (1967) have also noted that below a threshold concentration the filtration rates of *Pseudocalanus minutus*, *Euphausia pacifica*, and *Calanus pacificus* drop to zero. Cell size and morphology of the food influence filtration and ingestion rates in both laboratory and field situations. *Calanus finmarchicus*, *C. helgolandicus*, and *C. hyperboreus* preferentially filter the larger cells when offered a mixed culture of algae (Harvey, 1937; Fuller, 1937; Marshall and Orr, 1955, 1962; Mullin, 1963) and *P. minutus*, *E. pacifica*, *Acartia tonsa*, and *Temora longicornis* select certain segments of the size spectrum when offered natural phytoplankton (Geen and Hargrave, 1966; Sheldon and Parsons, 1967; Parsons et al., 1967).

At first glance it might appear that with some knowledge of a freshwater copepod's size and species; phytoplankton size, species, and concentration;

[1]Canadian Contribution to the International Biological Programme No. CCIBP-40.

Printed in Canada (J1544)

and climatic conditions, a good estimate might be made of the rates of filtration and ingestion. Almost all of our present knowledge comes, however, from marine studies and the small amount of freshwater work presently published indicates that the relationships between animal size, cell concentration, and filtration rate that might be derived from the marine studies are of little use when dealing with freshwater copepods (Richman, 1964, 1966).

The work reported in this paper was conducted to test the belief that freshwater copepods filter at a much lower rate than comparably sized marine copepods, and to study the filtration and ingestion rates of a freshwater copepod under conditions of varied cell type, size, and concentration. To achieve this end *D. oregonensis* Lilljeborg females and stage V copepodids were offered five pure cultures of algae in the laboratory, a mixed culture of diatoms in the laboratory, and natural phytoplankton in Marion Lake. *Diaptomus oregonensis* was chosen because a good deal of basic information is presently available on this species. F. H. Rigler (personal communication) has studied its population dynamics, Richman (1964, 1966) has done energy transformation work and experimented with cell concentrations and filtration rates, and Rigler and Langford (1967) and Sandercock (1967) have looked at the distribution and cogeneric occurrence in Ontario lakes.

METHODS

Five types of algae, all of which were found in Marion Lake (Dickman, personal communication), were used as potential food sources for *D. oregonensis* females and stage V copepodids. At all times the experiments were conducted at cell concentrations of about 20,000 cells per milliliter, so that changes in cell number could be accurately detected using a Coulter Counter and because Richman (1966) found that the filtration rates of *D. oregonensis* levelled off at about 20,000 cells per milliliter. The cells used were all obtained in pure culture from Dr J. Stein, Department of Botany, University of British Columbia, Vancouver, B.C. The species were: *Chlorella pyrenoidosa* (UBC 395), *Ochromonas* sp. (UBC 242), *Cryptomonas* sp. (UBC 12), *Chromulina scherffellii* (UBC 244), and *Chlamydomonas* sp. (UBC 66). The algal cells were grown under constant light conditions on soil medium at room temperatures and only cells coming from cultures that were in the log phase of growth were used in the experiments.

When pure cultures of algae were offered to *D. oregonensis* females and stage V copepodids the animals were removed from the lake and brought into the laboratory where they were held in the dark for 24 or 48 hr at lake temperature (about 17 C). From 8 to 12 hr before the experiment was about to begin the algal cells, which were to be used in the experiment, were suspended in lake water that had been filtered through a membrane filter with an aperture size of 0.80 μ. The algal water mixture was made up to a concentration approximating that desired for the experiment and was held in the dark at lake temperature so that cell division would slow down or stop. When the experiment was to begin a subsample of the algal–water mixture was run through a Coulter Counter and on the basis of these counts the algal–water mixture was diluted to the required concentration. A series of 65-ml bottles with ground-glass stoppers were filled with the algal–water mixture and 20 animals were placed in each of the experimental bottles. The control bottles had no animals. The bottles were then rotated on an 18-inch wheel at 0.25 rpm in the dark, at lake temperature, for 5 hr. At the end of this time the animals were removed from the experimental bottles and the algal cells in both the controls and the experimentals were counted using a Coulter Counter.

For experiments in which a mixture of diatoms were fed to *D. oregonensis* the methods outlined above were followed exactly. When *D. oregonensis* females and stage V copepodids were

fed lake phytoplankton the experimental method was as above except that the experiments were conducted at the lake in 100-ml bottles and the cells were settled and counted under an inverted microscope rather than with a Coulter Counter. Experiments in which *Cyclotella* and *Merismopedia* were tested as a food source were conducted in exactly the same manner as the natural phytoplankton experiments. *Diaptomus oregonensis* females and stage V copepodids were allowed to feed on an undiluted mixture of natural phytoplankton and their ability to remove *Cyclotella* and *Merismopedia* was tested by counting their numbers in the experimental and control bottles.

RESULTS

Filtration of a Unialgal Food Source

When five pure cultures of algae were offered to *D. oregonensis* females and stage V copepodids the filtration rates (Table 1) were within 1 SD on the sample mean of those found by Richman (1966) for adult *D. oregonnensis* feeding on *Chlorella vulgaris* and *Chlamydomonas reinhardti* concentrated at about 20,000 cells per milliliter. The accuracy of these estimates is substantiated by the fact that he used the C^{14} method to determine filtration rates whereas direct cell counts were used in this study. In general the filtration rates remained constant over the range of sizes and algal types that were used, but the ingested biomass increased with increased cell size (Table 1).

TABLE 1. Filtration rates of *D. oregonensis* in the laboratory. The filtration rates and fresh weight biomass per animal per day are given as the mean ± 1 SE on the mean.

Cell type	Mean cell vol in μ^3	No. of replicates	Cell concn in cells per ml	Filtration rate in ml of water per animal/day	Wet wt of cells ingested μg per animal/day
Chromulina scherfellii	122	9	2100±133	1.50±0.13	3.84±0.33
Chlorella pyrenoidosa	567	5	20700±147	1.33±0.22	15.61±2.58
Ochromonas sp.	905	6	20000±1224	1.68±0.11	30.41±1.99
Chlamydomonas sp.	2523	7	23000±755	1.43±0.11	89.98±6.38
Cryptomonas sp.	9985	6	19700±82	1.07±0.16	210.47±31.47

Filtration of a Mixed Food Source

Diaptomus oregonensis females and stage V copepodids were offered a mixed culture of diatoms of the genus *Navicula*. The diatoms did not come from Marion Lake but were obtained from D. Blinn (Department of Botany, University of British Columbia); however, six species of *Navicula* have been identified from Marion Lake. The diatoms, all of which were mixed together and used as a food source, ranged in volume from about 30 to 2000 μ^3. The filtering rate increased from 0 ml per animal per day for 30–60 μ^3 cells to a maximum of 2.07 ml per animal per day for 245–490 μ^3 cells (Table 2). Since the concentration of the small cells was near 20,000 cells per milliliter it might

TABLE 2. Results obtained when a mixture of *Navicula* diatoms was offered to *D. oregonensis* in the laboratory. Data are given as the mean ± 1 SE on the sample mean.

Range of vol in μ^3	30–60	61–122	123–244	245–490	491–980	981–1960
Mean cell vol in μ^3	45.0	91.5	183.5	367.5	735.5	1470.5
Control replication	5	5	5	5	5	5
Exptl replication	6	6	6	6	6	6
No. of cells per ml in controls	22675	17644	8581	3570	827	247
No. of cells eaten per animal/day	0.0±3525	4969±1061	11123±3793	7404±1233	1041±235	404±204
Mean filtering rate in ml per animal/day	0.00±0.15	0.28±0.06	1.29±0.44	2.07±0.34	1.26±0.28	1.63±0.82
Fresh wt biomass in μg per animal/day	0.00±0.16	0.45±0.09	2.04±0.69	2.72±0.45	0.76±0.17	0.59±0.30

be expected that the filtration rate would be about 1.5 ± 0.5 ml per animal per day as it was in the unialgal experiments. The fact that the filtration rate was 0.0 ml per animal per day indicates that *D. oregonensis* females and stage copepodids do not filter, or are incapable of filtering such small cells. The same might be said for the cells ranging from 61 to 122 μ^3. The biomass ingested increased rapidly with increased cell size up to a volume of 123 μ^3. Between 123 μ^3 and 490 μ^3 the biomass ingested remained high and relatively constant. Beyond 490 μ^3 the biomass ingested decreased with increased cell size (Table 2).

FILTRATION OF LAKE PHYTOPLANKTON

Lake phytoplankton that had been filtered through a plankton net with a mean aperture size of 70 μ was used as a food source during the summer of 1968. The results of the natural phytoplankton experiments suggested that, despite their size, very small particles of naturally occurring phytoplankton could be filtered by *D. oregonensis* females and stage V copepodids at rates comparable with those recorded when unialgal cultures (Table 1) and large diatoms (Table 2) were offered as food sources. This apparent contradiction strongly suggests that cell type must be an extremely important factor in the determination of filtration rates. This point is further substantiated by the fact that the rates of filtration on all of the natural phytoplankton size categories were higher than those recorded in either of the other two experiments.

There are, however, definite similarities between the natural phytoplankton and diatom results. In both instances the filtering rates underwent a rapid increase at approximately 100 μ^3, plateaued, and then gradually decreased

beyond approximately 800 μ^3. The ingestion rates showed similar increases at 100 μ^3 but decreased beyond 450 μ^3. The magnitude of the increases and decreases were different but the trends were very similar.

Cyclotella and *Merismopedia* were very common in Marion Lake during July and August of 1967 and 1968. During the summer of 1967, 10 experiments were run to test the hypothesis that *D. oregonensis* females and stage V copepodids were able to filter *Cyclotella antigua* and *C. glomerata* from the natural plankton of Marion Lake. It was found that the concentration of *Cyclotella* ranged from 67 to 144 cells per ml and the filtering rate was −1.84 ml per animal per day (±1 SE of 0.57 ml on the sample mean). These data imply that at Marion Lake the *Cyclotella* population was unaltered by *D. oregonensis* females and stage V copepodids. These data also apparently contradict the results of the previous experiments. Since *Cyclotella* in Marion Lake averaged only 15 μ diam they should have been eaten if size were the only factor influencing filtration rate. Their low concentration could also not have prevented them from being filtered because the larger naturally occurring phytoplanktons were concentrated at only 175 cells per milliliter and were filtered at a mean rate of 7.81 ml per animal per day. Again, these data suggest that cell type is very important in the determination of filtration rates.

Tests were also run to find out if *Merismopedia* was used by *D. oregonensis*. Four species (*Merismopedia elegans*, *M. punctata*, *M. glauca*, *M. tenuissima*) have been identified from the lake and have been found to occur in mats ranging from 15 to 30 μ in diameter. Six experiments similar to those conducted for diatoms suggested that the mats occurred at concentrations ranging from 502 to 681 mats per milliliter during August 1967 and that *D. oregonensis* females and stage V copepodids exhibited a filtration rate of −0.418 ml per animal per day (±1 SE of 0.85 ml on the sample mean) when fed *Merismopedia* along with all the other naturally occurring phytoplankton of Marion Lake. Because the mats tended to fragment if handled roughly it was extremely difficult to do meaningful experiments with this genus. It is possible that *D. oregonensis* females and stage V copepodids could have consumed some of the small cells that made up the mats. It is also possible that they may have broken small portions from the mats and ingested these. With the experimental method used it would have been impossible to detect either occurrence. At the moment it can only be said that despite their abundance mats of *Merismopedia* were not eaten by *D. oregonensis*.

DISCUSSION

It is apparent that the feeding behaviour of *D. oregonensis* females and stage V copepodids is extremely complex, and that the problems are by no means solved. The data derived from these experiments indicate that *D. oregonensis* is able to filter certain sized particles better than others. The *Cyclotella* and *Merismopedia* data indicate that some particles are not filtered despite the fact that their volumes and concentrations lie within the filterable ranges. The data also suggest that *D. oregonensis* acquires much of its energy

from the cells that range between 100 μ^3 and 800 μ^3. Cells smaller than 100 μ^3 occur in high concentrations but individually they are capable of adding little to the cumulative biomass ingested and are filtered at a relatively low rate. Cells larger than 800 μ^3 individually are able to add a great deal to the cumulative biomass ingested but are found in very low concentrations and are filtered at less than maximum rates.

The natural phytoplankton experiments indicated that the filtering rates in the lake were much higher than those previously recorded for freshwater copepods (Nauwerck, 1959; Comita, 1964; Richman, 1966). They were also higher (Table 3) than the rates recorded for *D. oregonensis* feeding on diatoms

TABLE 3. Results obtained when natural phytoplankton was offered to *D. oregonensis* at the lake. Data are given as the mean ± 1 SE on the sample mean.

Diam (μ)	2–2.8	2.9–5.7	5.8–8.6	8.7–11.5	11.6–14.4
Mean cell vol in μ^3	7.2	21.0	179.0	524.0	1150.0
Control replication	5	5	5	5	5
Exptl replication	12	12	12	12	12
No. of cells per ml in controls	7461±528	3591±320	1956±243	321±84	175±26
No. of cells eaten per animal/day	14304±9393	5352±4302	25248±3879	3600±845	1368±859
Mean filtering rate in ml per animal/day	1.91±1.25	1.49±1.20	12.90±1.98	11.21±2.63	7.81±4.90
Fresh wt biomass in μg per animal/day	0.10±0.07	0.11±0.09	4.51±0.69	1.88±0.44	1.57±0.99

of comparable sizes and concentrations (Table 2). These data certainly suggest that much more information must be gathered before *Diaptomus* feeding in natural situations can be fully understood. They also suggest that the animals are able to handle their natural food more efficiently than they can handle pure algal cultures and mixtures, grown under laboratory conditions.

Data from the literature make it possible to partially check the validity of the filtration rate data collected under natural phytoplankton conditions during the course of this work. Richman (1964) found that *D. oregonensis* used 2.160 ± 0.408 μ^1 of oxygen per copepod per day and he converted this to 0.0108 ± 0.002 calories output per animal per day at 22.0 ± 0.5 C using the conversion of Swift and French (1954). Using the oxygen consumption data of Comita (1968) and the same oxygen–calorie conversion, *D. oregonensis* females should use 0.0050 calories per animal per day at 15 C and 0.0077 calories per animal per day at 20 C. From these three determinations it is apparent that *D. oregonensis* females should use between 0.006 and 0.008 calories per animal per day at 18 ± 1.0 C, which is the temperature that the

Marion Lake animals experienced. The data indicate that the Marion Lake animals ingested about 8.17 μg wet weight of phytoplankton per animal per day when fed naturally occurring plankton. Using the method of conversion of Richman (1964) this represents an intake of 0.0045 calories per animal per day. So that even at the very high filtration rates recorded in this study *D. oregonensis* females would use more calories than they were taking in and the above calculations make no provision for egg production.

At least two additional sources of food were not accounted for in this study and both would tend to increase the number of calories ingested. During the course of the natural phytoplankton experiments cells larger than 1400 μ were not concentrated enough to be included in the feeding experiments despite the fact that some cells of this type were observed in the water. Since the animals did ingest a significant proportion of the largest cells counted and since the unialgal experiments indicated that they were capable of ingesting very large cells it might be assumed that the animals remove at least some of the large cells they encounter. Detritus–bacteria particles may also be filtered by *D. oregonensis*. Naurwerck (1963) estimated that algal production in Lake Erken during July was only two to three times greater than the *Eudiaptomus* production. At these rates he calculated that *Eudiaptomus* could never have sustained their high July growth rates if they hadn't been drawing upon other food resources such as detritus. It is very possible that the Marion Lake *D. oregonensis* also draw upon detritus and their accompanying bacteria in their attempt to balance their energy budget.

ACKNOWLEDGMENTS

I would like to acknowledge Dr J. Stein and D. Blinn both of whom supplied algal cultures, and M. E. Jermolajev who identified the zooplankton. I must also extend a special thanks to Dr I. E. Efford whose help and encouragement contributed greatly to both the research and manuscript.

REFERENCES

COMITA, G. W. 1964. The energy budget of *Diaptomus siciloides*, Lilljeborg. Verh. Int. Ver. Limnol. 10: 643–653.
1968. Oxygen consumption in *Diaptomus*. Limnol. Oceanogr. 23: 51–57.

CONOVER, R. J. 1956. Oceanography of Long Island Sound, 1952–54. VI. Biology of *Acartia clausi* and *A. tonsa*. Bull. Bingham Oceanogr. Collect. Yale Univ. 15: 156–233.

FULLER, J. L. 1937. Feeding rate of *Calanus finmarchicus* in relation to environmental conditions. Biol. Bull. (Woods Hole) 72: 233–246.

GEEN, G. H., AND B. T. HARGRAVE. 1966. Primary and secondary production in Bras d' Or Lake, Nova Scotia, Canada. Verh. Int. Ver. Limnol. 16: 333–340.

HARVEY, H. W. 1937. Note on selective feeding by *Calanus*. J. Mar. Biol. Ass. U.K. 22: 97–100.

MARSHALL, S. M., AND A. D. ORR. 1955. Studies on the biology of *Calanus finmarchicus*. VIII. Food uptake, assimilation, and excretion in adult and stage V *Calanus*. J. Mar. Biol. Ass. U.K. 34: 495–529.
1962. Food and feeding in copepods. Rapp. Proces-Verbaux Reunions Cons. Perma. Int. Explor. Mer 153: 92–98.

MULLIN, M. M. 1963. Some factors affecting the feeding of marine copepods of the genus *Calanus*. Limnol. Oceanogr. 8: 239–250.

NAUWERCK, A. 1959. Zur Bestimmung der Filtrierrate limnischer Planktontiere. Arch. Hydrobiol. Suppl. 25: 83–101.

1963. Die Beziehungen Zwischen Zooplankton und Phytoplankton im See Erken. Symb. Bot. Upsal. 17: 1–163.

PARSONS, T. R., R. J. LEBRASSEUR, AND J. D. Fulton. 1967. Some observations on the dependence of zooplankton grazing on the cell size and concentration of phytoplankton blooms. J. Oceanogr. Soc. Jap. 23: 10–17.

RICHMAN, S. 1964. Energy transformation studies on *Diaptomus oregonensis*. Verh. Int. Ver. Limnol. 15: 654–659.

1966. The effect of phytoplankton concentration on the feeding rate of *Diaptomus oregonensis*. Verh. Int. Ver. Limnol. 16: 392–398.

RIGLER, F. H., AND R. R. LANGFORD. 1967. Cogeneric occurrences of species of *Diaptomus* in Southern Ontario Lakes. Can. J. Zool. 45: 81–90.

SANDERCOCK, G. A. 1967. A study of selected mechanisms for the coexistence of *Diaptomus* spp. in Clarke Lake, Ontario. Limnol. Oceanogr. 12: 97–112.

SHELDON, R. W., AND T. R. PARSONS. 1967. A practical manual on the use of the Coulter Counter in marine science. Coulter Electronics Sales Company-Canada, Toronto, Ont. 65 p.

SWIFT, R. W., AND C. E. FRENCH. 1954. Energy metabolism and nutrition. Scarecrow Press, Washington, D.C. 264 p.

Toxicity of Yellow Phosphorus to Herring (*Clupea harengus*), Atlantic Salmon (*Salmo salar*), Lobster (*Homarus americanus*), and Beach Flea (*Gammarus oceanicus*)

V. Zitko, D. E. Aiken, S. N. Tibbo,
K. W. T. Besch, and J. M. Anderson

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

Zitko, V., D. E. Aiken, S. N. Tibbo, K. W. T. Besch, and J. M. Anderson. 1970. Toxicity of yellow phosphorus to herring (*Clupea harengus*), Atlantic salmon (*Salmo salar*), lobster (*Homarus americanus*), and beach flea (*Gammarus oceanicus*). J. Fish. Res. Bd. Canada 27: 21–29.

Yellow phosphorus is extremely toxic to aquatic life. In herring, salmon, and lobster, the toxic effect of phosphorus is irreversible and probably cumulative. For herring LT_{50} (hours) = $300/C^{0.87}$, where C = μg/liter of phosphorus. No clear indications of an incipient lethal level were found, an LT_{50} being obtained even at a concentration of 2.5 μg/liter. Incipient lethal levels of yellow phosphorus for lobster, salmon, and beach flea are 40 μg/liter, 18 μg/liter, and 3–4 mg/liter, respectively. Poisoned fish turn red and show signs of extensive hemolysis. Blood of lobster congeals, and after death the thorax may be filled with thick gel and the heart grossly distended. For lobster, LT_{50} (hours) = $300–0.17x$, where x = product of phosphorus concentration and exposure time (μg days/liter). The oxidation of dispersions of yellow phosphorus in water is kinetically a first-order reaction with half-life of 2–7.5 hr. The adsorption of yellow phosphorus on a solid support such as bottom mud substantially decreases the rate of oxidation.

Received September 17, 1969

INTRODUCTION

Although the toxicity of yellow phosphorus to mammals has been studied extensively (Hamilton and Hardy, 1949), the only work to our knowledge on fishes has dealt with bluegills, *Lepomis macrochirus* (Isom, 1960). For bluegills the reported TL_m[1] values ranged from 0.105 mg/liter at 48 hr to 0.025 mg/liter at 163 hr. However, concentrations of yellow phosphorus were calculated from amounts added to the system. In view of the relative ease with which phosphorus is oxidized, the actual effective concentrations were probably much lower than those reported.

Recently in Long Harbour (a small arm on the east side of Placentia Bay), Newfoundland, a large accidental pollution by yellow phosphorus occurred. Massive fish mortalities, particularly by herring, *Clupea harengus*, were observed. This incident, together with the general dearth of information on the toxicity of yellow phosphorus to aquatic forms, prompted the present study.

[1]TL_m = median tolerance limit, concentration of toxicant causing 50% mortality in a given time.

Printed in Canada (J1592)

MATERIALS AND METHODS

Fish

Herring (10–15 cm total length, age-group 1) were obtained from commercial weirs in nearby Passamaquoddy Bay, New Brunswick. They were seined from the weirs by our own technicians who handled the fish with extreme care (to avoid loss of scales or other injury). The time from weir to laboratory tank was about 1 hr. At no time during the operation were the fish removed from water. Holding procedures in the laboratory were essentially the same as those described by Das and Tibbo (1962). The fish were not fed and although there was an obvious loss of condition (fatness), they remained healthy for at least a month when they were replaced with a new supply.

Atlantic salmon parr, *Salmo salar* (7–13 cm total length) were obtained from local hatcheries of the Department of Fisheries and Forestry of Canada. Acclimation and feeding followed standard practice (Sprague, 1968).

Beach fleas, *Gammarus oceanicus*, were collected in local tidal zones and were held for 6 days in sea water in a seaweed-filled, aerated tank prior to experimental use.

Small male and female lobsters, *Homarus americanus* (mean carapace length 59.6 mm), collected at Miminegash, Prince Edward Island, had been held for 10 months in running sea water in our laboratory before experimental use. They were fed twice weekly.

No feeding of any of the test animals occurred during the test periods.

Bioassay

All bioassays were static tests. For herring and salmon, 10 fish were used per test. Testing was done in 70 × 45 × 30 cm high plastic tanks containing approximately 60 liters of continuously aerated sea water (*p*H 8, salinity 30‰, temperature 7–9 C) or fresh water (*p*H 6.9–7.2, total hardness 13–16 mg/liter $CaCO_3$, temperature 12–13 C). All tanks were inspected every hour around the clock.

For beach fleas, plastic containers (8 × 8 × 13 cm high) filled with 3 g of sand and 200 ml of salt water at 18–21 C were used. Five or six specimens were put into the container immediately after the preparation of the test solution. Two series of experiments were carried out, one in still water, the other in moving water (although no turbulence occurred) arranged by putting the containers on a moving platform. In neither instance was aeration provided. Each experiment was replicated at least once.

Tests with lobsters were performed in 1 × 1 × 0.3 m high plastic tanks holding 10 lobsters in 200 liters of continuously aerated sea water. Phosphorus-contaminated bottom mud was stirred at least once daily. Suspensions containing 0.5, 1.0, and 10% of contaminated mud were used.

LT_{50}'s (median time for death of 50% of the population in question) were determined from the line of best fit describing the relation between percent mortality and exposure time, using probit and log scales respectively (see Sprague, 1969).

Phosphorus Preparations

Four types of preparations containing yellow phosphorus were used: phosphorus-contaminated bottom mud from the scene of the pollution incident; "phossy water" — a supernatant of colloidal phosphorus from condensation and storage processes involved in the commercial production of crude yellow phosphorus; an aqueous dispersion (laboratory-made) of yellow phosphorus; and mud, sand, or alumina (Al_2O_3), artificially contaminated by yellow phosphorus.

The aqueous dispersion was prepared by adding a saturated solution of yellow phosphorus in ethanol to distilled water. Distilled water (1 liter) was deoxygenated by a stream of nitrogen and the solution of phosphorus (5 ml) was added by a pipet discharged below the water surface under vigorous stirring. The resulting dispersion contained 9–12 mg/liter of phosphorus. Aliquot amounts were added to the bioassay tanks.

Artificial contamination of the various substrates mentioned above was carried out as follows: yellow phosphorus was dissolved in carbon disulfide, the dry substrate was suspended in carbon tetrachloride, and an aliquot of the phosphorus-containing solution was added. The mixture was homogenized on a rotatory evaporator for 15 min and the solvent was evaporated

in vacuum at 30 C. The dry residue, generating white fumes on contact with air, was covered by a layer of distilled water. Finally, varying amounts of the settled material were weighed out and used in the bioassay.

Determination of Yellow Phosphorus

Water samples (2 liters each), taken from the bioassay tanks, were extracted with benzene (40 ml) by shaking in a separatory funnel for 3 min. The benzene layer was separated, centrifuged to break the emulsion, and washed with a small amount of distilled water. Bromine (2% solution in carbon tetrachloride) was added to the benzene extract until the colour persisted. Distilled water (15 ml) was then added and the mixture was stirred for 15 min on a magnetic stirrer. The aqueous phase was separated, the flask and the benzene layer were washed with distilled water (9 ml). The combined aqueous solutions were adjusted to 25 ml and phosphate was determined by the standard molybdate-stannous chloride method (American Public Health Association, 1965).

RESULTS

Stability of Yellow Phosphorus in Water

The rate of oxidation of yellow phosphorus, which was studied most extensively in the fish bioassay tanks, is a first-order reaction (Fig. 1). The half-life of yellow phosphorus is 2, 7.5, and 20 hr in aqueous dispersion, diluted "phossy water," and in 1% suspension of phosphorus-contaminated bottom mud, respectively. In the lobster tanks, which contained phosphorus-contaminated mud, the decrease of phosphorus concentration was less pronounced and quite variable. After 14 days of constant aeration, from 1 to 50% of yellow phosphorus was oxidized. No comparable measurements were made during the beach flea experiments.

Toxicity to Herring

The relation between the time in hours to 50% mortality (LT_{50}) for herring, and the initial concentration $(C)^2$ in µgrams/liter of phosphorus is shown in Fig. 2. The data can be described by the equation:

$$LT_{50} = \frac{300}{C^{0.87}}.$$

Evidently the incipient lethal concentration is less than 2.5 µg/liter since at this dose, the lowest used, an LT_{50} (of approx 130 hr) was obtained.

Of some interest were results that showed that the LT_{50}'s were almost the same both for herring exposed to a suspension of the phosphorus-contaminated mud for 3 hr and then transferred into clean running water, and for herring kept constantly in the suspension. The toxic action of phosphorus, at least at relatively high levels, appears to be irreversible and perhaps cumulative.

The most noticeable effect of phosphorus poisoning on herring was that they turned red. In early stages a slight reddish tinge appeared at the base

[2]Concentration extrapolated to $t = 0$.

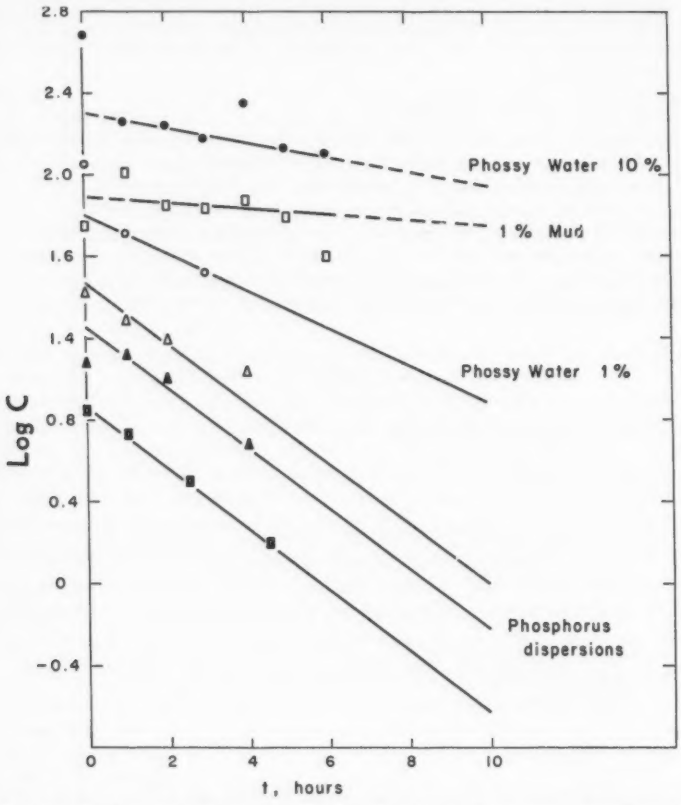

FIG. 1. Kinetics of oxidation of yellow phosphorus. C = concentration of yellow phosphorus, μgrams/liter.

of the pectoral, pelvic, and dorsal fins. As exposure time and concentrations of the pollutant increased, the red colour increased in intensity and spread to other parts of the body. Before death the eyes, operculum, and snout became bright red and there was some redness in all external areas.

After death the herring were examined for internal damage and pieces of gill, liver, kidney, spleen, and intestine were preserved for subsequent histological examination. Although this study has not yet been completed, there were obvious symptoms of extensive hemolysis. The gills were pink instead of bright red as in normal fish. Both the liver and spleen were abnormally pale and the intestine and body cavity frequently contained a pink, yellow, or green fluid. The blood was pale and hematocrit values approached zero (37–40% for normal fish).

The physical effects of phosphorus poisoning on herring were not accompanied by changes in behaviour. Until a short time before death, there

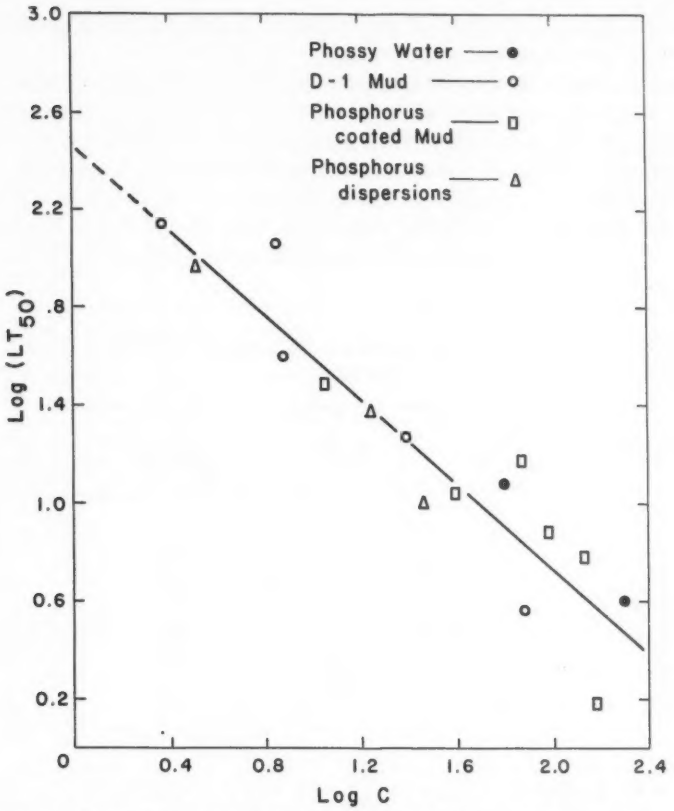

FIG. 2. Toxicity of yellow phosphorus to herring (*Clupea harengus*). LT_{50} = time to 50% mortality, hours; C = concentration of yellow phosphorus extrapolated to $t = 0$, μgrams/liter. D-1 Mud = bottom mud from the scene of the pollution incident.

was no apparent disorientation or loss of balance. Unless disturbed, they maintained a schooling formation and swam slowly around the sides of the tanks. As death approached, however, swimming speed increased erratically, and the fish swam individually in circular or spiral fashion with heads pointed upwards.

TOXICITY TO SALMON

Phosphorus poisoning of salmon resulted in symptoms similar to those observed in herring. However, salmon appear to be more resistant to phosphorus. The incipient lethal level is approximately 18 μg/liter.

TOXICITY TO BEACH FLEA

Because of the small volumes of the bioassay containers, it was not possible to determine the actual concentration of phosphorus in the beach flea experi-

ments. Concentrations were calculated from the amount added to the system. The results are summarized in Table 1.

Incipient lethal levels are 3–4 mg/liter of phosphorus in still water, and about 6.5 mg/liter in moving water. Lethal doses of yellow phosphorus paralyze beach fleas almost immediately. The animals become stretched out, or slightly bent, and do not respond well to stimuli, making a precise assessment of death difficult and not very exact. All animals exposed to 8 mg/liter of yellow phosphorus for 3 hr recovered when transferred into clean water. Recoveries of 90 and 20% of test animals were observed after 1 hr exposure to 17 and 40 mg/liter of yellow phosphorus.

TABLE 1. Toxicity of yellow phosphorus to beach flea (*Gammarus oceanicus*).

	In moving water after 24 hr		In still water after 24 hr	
Estimated amount of P in mg/liter	% paralyzed	% dead	% paralyzed	% dead
1	0	0	0	0
2	0	0	0	0
3.2	0	0	10	0
4.0			90	90
4.5	0	0	100	100
6.0	100	30		
6.5	20	5	100	100
8.0			100	100
9.0	100	75	100	100
10.0	100	100		
12.0			100	100

TOXICITY TO LOBSTER

The first experiment with lobsters quickly established that, as was shown for herring, the toxic effects of phosphorus appear to be irreversible. Lobsters exposed to contaminated mud and then transferred to clean water after 167 hr died at the same time as others that remained in continuous contact with the contaminant. LT_{50}'s in both instances were 265 hr.

Nine additional experiments were conducted to explore the relation between exposure duration and phosphorus concentration (Table 2). Concentrations (μgrams/liter were determined 24 hr after the phosphorus-contaminated mud was mixed with water. Exposure time refers to duration (days) in contaminated mud before changing to clean water. The time-concentration index (third column) represents the product of exposure in days times the determined concentration. The data clearly indicate that LT_{50} is related more to a time-concentration effect than to either time or concentration alone. For example, 4 days at 120 μg/liter produces an LT_{50} almost identical to that for 7 days at 50 μg/liter. Comparison of the time-concentration index (μgram days/liter) with LT_{50} reveals a highly significant correlation (0.954), and the regression of this relationship is given in Fig. 3.

TABLE 2. Time-concentration effect of phosphorus toxicity to lobster (*Homarus americanus*).

Exposure days	μg/liter	μg days/liter	Hr to 50% death	Lethal range hr
7	120	840	149	137–173
7	80	560	205	196–258
9	60	540	215	206–291
4	120	480	235	209–307
7	50	350	239	180–349
7	40	280	240	196–309
7	20	140	[a]	–
2	40	80	[a]	–
7	10	70	[a]	–

[a]No mortality in 60 days.

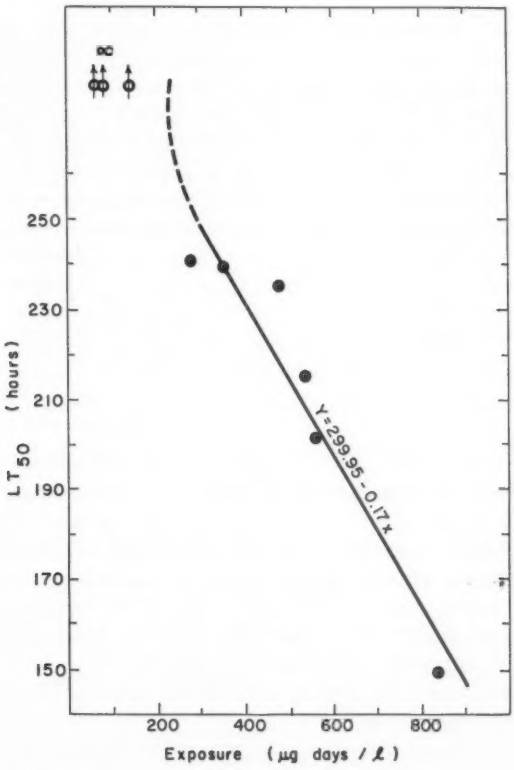

FIG. 3. Relation between product of exposure time and phosphorus concentration and time to 50% mortality for lobster (*Homarus americanus*).

The time-concentration phenomenon is consistent with our hypothesis that the toxic action of phosphorus is irreversible. This is further supported by an experiment in which 10 lobsters were placed in a mud suspension containing 50 μg/liter of yellow phosphorus. Of these, 5 remained in continuous contact with the contaminant, and the other 5 spent alternate 24-hr periods

in clean, flowing water. Those in continuous contact had an LT_{50} of 239 hr, and those in alternate 24-hr periods had an LT_{50} of 240 contact hr (480 elapsed hr). Water analysis revealed no significant change in the level of phosphorus in the first 14 days of the experiment.

The incipient lethal level of yellow phosphorus is not clearly defined by these experiments (Table 2), but the data do indicate that it lies between 20 and 40 μg/liter.

Lobsters exposed to lethal levels of yellow phosphorus begin to show symptoms at least 12 hr before death. They lose muscle tone, become lethargic, move the pereiopods in a jerky, uncoordinated and aimless manner, and eventually lose all neuromuscular response. At this point they were considered dead, although rhythmic cardiac contractions continued for an additional 1–3 hr.

Postmortem examination revealed that the blood of afflicted lobsters had coagulated to varying degrees. In lobsters examined prior to total loss of neuromuscular response, the blood was thick but not congealed. By the time of death the blood had formed a gel in the immediate vicinity of the heart and stomach. By 6 hr post-mortem the blood throughout the cardiogastric region was a thick gel and, in some instances, the heart was grossly distended. This condition has also been observed in lobsters given a lethal dose of isobutyl alcohol (often used as an anaesthetic).

There is no conclusive evidence that sublethal exposure to yellow phosphorus adversely affects the molt cycle. Lobsters held for 2 days at 40 μg/liter proceeded through the various stages of proecdysis at the expected rate, and two others that experienced 7 days at 20 μg/liter completed a molt 14 days later with apparently normal postmolt cuticular mineralization.

DISCUSSION

In spite of the high reactivity of yellow phosphorus, our results show that when dispersed in water it is surprisingly stable, particularly when it is adsorbed on a solid support such as mud. The oxidation rates depend, of course, upon the amount of dissolved oxygen present and in our experiment these rates were probably near maximum since in almost all instances aeration insured that oxygen levels were at or near saturation. In nature, oxidation rates would almost certainly be lower than in our experiments. Bottom mud, for example, would be typically low in oxygen. This means that areas contaminated by yellow phosphorus would likely remain so polluted for considerable time.

Apart from demonstrating the extreme toxicity of yellow phosphorus to herring, salmon, lobster, and beach flea (in that order), perhaps the most significant results were those demonstrating the apparent irreversibility of the toxic effects. This is of considerable ecological importance. Although accidental pollution by yellow phosphorus may be confined to a relatively small area, the biological consequences may occur over a much wider area. For example, if herring (a migratory species) should encounter a phosphorus-

contaminated area and absorb a lethal load as they passed through it, they might not actually be killed until they were far removed from the polluted area. This was probably the situation in Placentia Bay, Newfoundland, where massive herring mortalities were observed up to 60 miles from the localized pollution site in Long Harbour.

The most logical cause of death of the fish species used in these experiments was asphyxiation brought on by massive hemolysis. Asphyxiation may also have been the cause of the lobster mortalities since presumably the observed thickened blood would be too viscous to circulate freely through the gills. Uncontaminated lobsters that were held in oxygen-free water for 24 hr exhibited all the symptoms leading to death previously observed in the phosphorus-poisoned ones (although the blood, of course, remained thin and showed no tendency to congeal).

ACKNOWLEDGMENTS

We wish to thank Dr D. R. Idler, Fisheries Research Board of Canada, Halifax Laboratory, Halifax, N.S. for bottom mud samples from Long Harbour, Newfoundland; Dr D. H. Steele, Memorial University, St. John's, Nfld., for kind cooperation; all our co-workers who did not hesitate to work far beyond their duties on this urgent and hazardous project; W. G. Carson, R. J. McNabb, R. C. Murray, and T. Foulkes for skillful assistance in the bioassay tests; W. V. Carson and Barbara J. Finlayson for chemical analyses; Peter Vass, Barbara Barto, Dave Younker, Gerry Hare, and Ruth Cantin for the preparation of samples for histology; and Madelyn M. Irwin for collecting and up-dating the records.

REFERENCES

American Public Health Association. 1965. Standard methods for the examination of water and wastewater including bottom sediments and sludges. 12th ed. APHA, Inc., New York.

Das, N., and S. N. Tibbo. 1962. On the feeding and growth of young herring (*Clupea harengus* L.) in captivity. J. Fish. Res. Bd. Canada 19: 981–983.

Hamilton, A., and H. L. Hardy. 1949. Industrial toxicology. 2nd ed. New York.

Isom, B. G. 1960. Toxicity of elemental phosphorus. J. Water Pollut. Contr. Fed. 32: 1312–1316.

Sprague, J. B. 1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. Water Res. 2: 367–372.

1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. (In press.)

Alterations in the Electrophoretic Protein Patterns of Refrigerated Fish

G. S. MOORE,[1] H. A. PETERS, AND R. E. LEVIN

Department of Public Health
University of Massachusetts, Amherst, Mass. 01002, USA

MOORE, G. S., H. A. PETERS, AND R. E. LEVIN. 1970. Alterations in the electrophoretic protein patterns of refrigerated fish. J. Fish. Res. Bd. Canada 27: 31–38.

Expressible fluid was obtained from fillets on the 1st, 4th, and 14th days of refrigeration. The protein concentration was determined and electrophoresis using cellulose polyacetate strips was performed on each sample. The protein concentration remained constant between 6 and 8 g/100 ml over the 2-week period. Qualitative and quantitative examination of the densitometry tracings revealed that protein(s) having specific migratory characteristics were strongly associated with the duration of storage at 2 C.

Received August 12, 1969

INTRODUCTION

THE SPOILAGE OF FISH is a complex process, and the pattern of spoilage is dependent on a number of factors such as handling, storage, diet, feeding grounds, stress at death, and processing (Stansby, 1958, 1963). No single test to date has been developed that yields a reliable measure of the freshness of fish (Gould, 1965; McKee et al., 1963; Stansby, 1958, 1963; Tarr, 1954). However, distributors and buyers of fish continue to be concerned with shelf life and need standards and tests that will indicate spoilage and relative freshness (Gould, 1965). Stansby and other authors feel that tests for freshness based upon measurement of existing chemical and bacterial indices are not widely applicable because of the extremes in variation of the fish (Gould, 1965; Stansby, 1958, 1963). It is felt that more promising results may be obtained from the study of enzyme kinetics or enzymatic degradation taking place within the flesh of refrigerated fish. Gould (1965) maintains that electrophoretic properties of the fish may change as a result of enzyme degradation or the migration of enzymes through postmortem tissues. Animals as well as fish have been classified by the electrophoretic patterns of their proteins (Hutten, 1960; Wilson, 1967). Since it is possible to classify animals by their enzymes and other proteins (Gould, 1965), then it should be possible to trace the loss in quality of animal or fishery products by the changes in the electrophoretic properties of tissue proteins. This study attempts to answer the following questions: do protein patterns become altered as the fish ages and deteriorates; and if so, can the shelf life of a fillet be predicted from its electrophoretic pattern?

[1]Present address: 605A Hibbard Drive, Chapel Hill, North Carolina 27514, USA.

Printed in Canada (J1566)

MATERIALS AND METHODS

Preparation of fillets — A total of eight haddock fillets were tested. Four skinned fillets of uniform size and age were obtained from the Pier Fish Company (Boston, Mass.) and tested in January. The remaining four fillets were not uniform in size and age, and were obtained with the skin intact from a local commercial source and tested in March. Half of each fillet was first frozen at −20 C and then placed with the other halves at 2 C. Because the pattern of spoilage is dependent on a number of factors such as the presence or absence of skin, freezing, the season, and differences in size and age between individual fillets, these variables were studied to determine if they would mask changes in the electrophoretic patterns as a function of storage at 2 C. Fish muscle press juice was prepared by removing 20 g of skeletal muscle near the midsection of the fillet and macerating the tissue in a Nalgene centrifuge tube with a scalpel. Samples were then centrifuged for 10 min at 27,000 *g* in a model RC-2 Sorvall centrifuge at 2 C. The electrophoretic pattern and protein concentration of the expressible fluid (fish extract) was determined for all samples on the 1st, 4th, and 14th day after arrival.

Electrophoresis — The protein concentration of the fish extract was determined for each sample using a standard Biuret reaction with Hycel Biuret reagent (Hycel Inc., Houston, Texas) and reading the optical density at 540 nm in a Spectronic 20 colorimeter. Electrophoresis was performed with a Gelmann apparatus (Gelmann Inst. Co., Ann Arbor, Michigan) using Sepraphore III cellulose acetate strips (2.5 × 15 cm) and 0.05 M tris (hydroxymethyl) amino methane barbital buffer at *p*H 8.6 (Hartmann-Ledden Co., Philadelphia, Pennsylvania). A potential of 0.05 ma/cm² was applied for 60 min. Strips were then stained in Ponceau S dye (Hartmann-Ledden Co.) for 10 min, washed several times in 5% acetic acid, and then cleared for densitometry by rinsing twice with absolute ethanol (1:9 v/v). Densitometry tracings were performed with a model B4800 Densicord coupled with an R337 Integraph automatic integrator (Photovolt Corp., New York City).

For the purpose of this study, each of four major bands which appeared on the strips was arbitrarily designated by a letter (Fig. 1). The slowest migrating band was designated as A, and the more rapidly migrating bands were designated B, C, and D respectively. Densitometry tracings of the electropherograms converted the relative densities and positions of the bands into graphic form (Fig. 2). The percentage of total protein represented by each protein band was calculated from the tracings of the automatic integrator. Standardization of the equipment and determination of the range of error for the system was determined by electrophoresis of Monitrol, a freeze-dried control prepared from human blood (Dade reagents, Miami, Florida). Bands which approximated 60% of the total protein had a range of allowable variation (±2 SD) of ±1.8% and values that approximated 11% had a range of allowable variation of ±0.64%.

When major bands were not completely separated, approximations were made by drawing a line parallel to the Y-axis through a point at which the curve deviated from symmetry thus dividing the fraction into two bands.

RESULTS

Protein Concentrations of Fish Extract

Table 1 lists the protein concentrations (g/100 ml) of fish extracts from all samples not subjected to prefreezing. Data for the prefrozen samples was excluded since they were portions of the same fillets. It can be seen that the protein concentrations of the fish extracts fall within a range of 6–8 g/100 ml with very little difference in the protein concentration from 1 day to 14 days of storage. The freshness of the fillets was exhausted after 6 days, and they had undergone advanced deterioration after 14 days. The protein concentrations of the fish extracts did not vary significantly with storage time at 2 C.

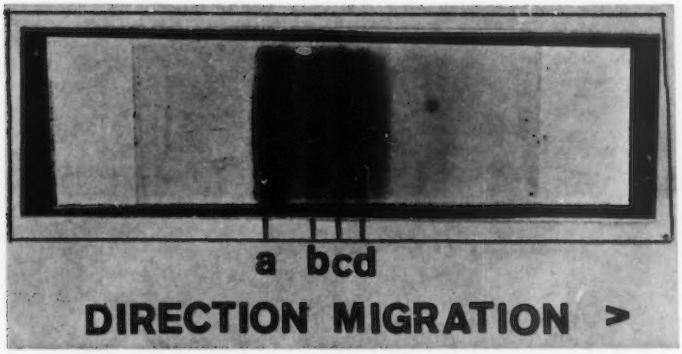

FIG. 1. Electropherogram of fish muscle press juice showing labeling for the protein bands *a*, *b*, *c*, and *d*.

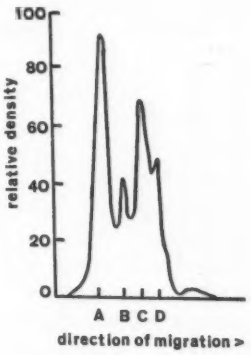

FIG. 2. Densitometry tracing of electropherogram shown in Fig. 1.

TABLE 1. Protein concentration (g/100 ml) for fish muscle press juice prepared from nonfrozen fillets after 1, 4, and 14 days of refrigeration at 2 C.

Month tested	Sample no.	Protein concentration (*g/100 ml*) Duration of storage at 2 C (*days*) 1	4	14
Jan.	1	7.5	7.3	5.8
	2	7.5	6.8	6.6
	3	6.7	6.5	7.4
	4	7.7	7.4	8.0
Mar.	5	8.0	8.2	7.7
	6	6.6	7.1	6.4
	7	7.4	8.0	7.9
	8	7.0	7.0	6.1

ELECTROPHORESIS: QUALITATIVE DIFFERENCES

Eight separate fillets were tested. Half of each fillet was prefrozen at −20 C, then stored with the other halves at 2 C prior to electrophoretic studies. For skinned fillets, prefreezing greatly influenced the electrophoretic patterns when compared with the nonfrozen halves (Fig. 3). With fillets having the skin intact, prefreezing did not influence the patterns (Fig. 4). The patterns were found to vary markedly between individual fish samples when they were not of uniform size and age (Fig. 5), but were remarkably similar when the fillets were uniform (Fig. 6). However, it was still possible to observe certain consistent changes in the densitometry tracings as a function of storage time. The most significant changes in the electrophoretic patterns occurred with the B band (Fig. 7). In most instances there was a marked tendency for the B band to graphically disassociate from the C band toward the A band.

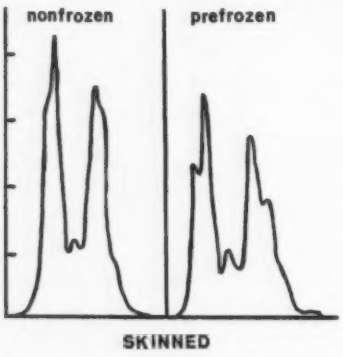

FIG. 3. Comparison between densitometry tracings of prefrozen and nonfrozen portions of a skinned fillet.

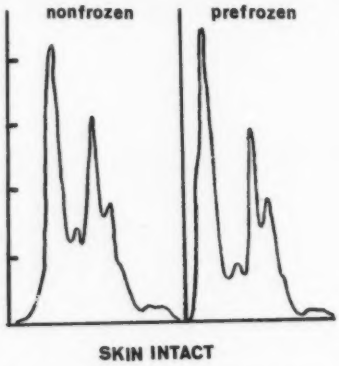

FIG. 4. Comparison between densitometry tracings of prefrozen and nonfrozen portions of a fillet with its skin intact.

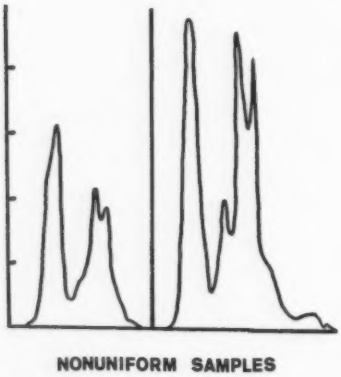

FIG. 5. Comparison between densitometry tracings of fillets not uniform in size and age.

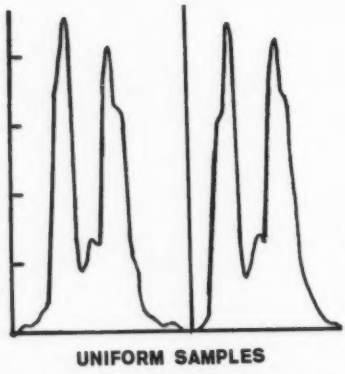

FIG. 6. Comparison between densitometry tracings of fillets uniform in size and age.

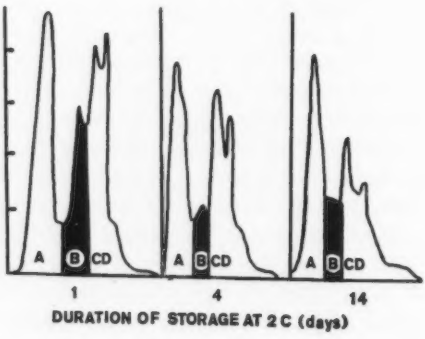

FIG. 7. Comparison between densitometry tracings prepared from a single fillet stored at 2 C for 1, 4, and 14 days.

Electrophoresis: Quantitative Differences

Table 2 lists the percentage of total protein represented by each protein band (A, B, C, and D) for all samples. Of the protein measured in the fish press juice, 40–50% migrated in the A-bands and this represented the largest percentage migrating in any one band. Between 4.5 and 15.7% of the protein migrated in the B band with an average between 9 and 10%. This represented the smallest percentage of protein migrating in any band. Between 40–50% of the protein migrated in the C and D bands collectively, and this amount was often evenly divided between the two bands. From analysis of variance with a split plot design (Stal, 1960), the B band alone was found to vary significantly with duration of storage at 2 C. To study this further, the percentage areas for the B bands for all samples with their skin intact were graphed as a function of storage time 2 C (Fig. 8). For nonfrozen samples with the skin intact, the percentage of protein migrating in the B bands decreased from the 1st to the 4th day of storage and increased from the 4th to the 14th. This type of behavior was shown to be statistically significant.[2]

A "P" value of 0.0039 strongly supports the conclusion that changes occurred in the B band as a function of storage time for nonfrozen fillets with the skin intact. Prefrozen samples demonstrated three (+, −) and one (−, −) combinations. Therefore, $X \rightarrow B(4, 0.25)$ and Pr $(X = 3)$ is equal to 0.047. Here again, the statistical evidence supports the conclusion that for prefrozen samples with the skin intact, changes occurred in the B band as a function of storage time.

The previous analysis referred to fillets that had their skin intact. When similar data was graphed for these samples without skin, different patterns of behavior could be seen (Fig. 9). All prefrozen skinned samples demonstrated (+ , −) combinations that were typical for the samples with the skin intact. Consistent with the above argument, the Pr $(X = 4)$ is equal to 0.0039, which statistically supports the contention that the percentage of protein migrating in the B band changed as a function of storage time for prefrozen skinned samples. The nonfrozen skinned samples behaved quite differently than all the others. Three of the samples were (−, −) combinations indicating that the percentage of protein migrating in the B band increased from day 1 to day 4 and then increased again from day 4 to day 14. This difference in behavior could possibly have been associated with the absence of skin as this tended to be an important factor associated with changes in the electrophoretic

---

[2]Let D_{1j} = the percentage of protein migrating in the B band for the jth sample after 1 day of storage; D_{4j} = the percentage of protein migrating in the B band for the jth sample after 4 days of storage; D_{14j} = the percentage of protein migrating in the B band for the jth sample after 14 days of storage; and Y_j = sign of $(D_{1j} - D_{4j})$ and Z_j = sign of $(D_{4j} - D_{14j})$. Thus four possible combinations exist for (Y_j, Z_j). These are (+, +), (−, −), (+, −), and (−, +). If the null hypothesis states that there is no directional tendency for either the day 1 to day 4 or day 4 to day 14 changes, then the four combinations are equally likely assuming the changes are independent. Hence, Pr $[(+, -)/H_0] = 0.25$. Let X = the number of (+, −) combinations for $n = 4$ independent samples, then $X \rightarrow B(4, 0.25)$ and the $Pr(X = 4)$ is given by $P4 = 4!/4!\,(0.25)^4\,(0.74)^0 = 0.0039$.

TABLE 2. Percentage of total protein represented by each protein band in electropherograms of fish muscle press juice prepared from all samples after 1, 4, and 14 days storage at 2 C.

Month tested	Group	Sample no.	% total protein 1 day at 2 C — Protein bands A	B	C	D	4 days at 2 C — Protein bands A	B	C	D	14 days at 2 C — Protein bands A	B	C	D
Jan.	Non-frozen	1	44.0	8.0	26.5	21.5	39.0	11.0	25.2	24.8	41.8	10.7	26.2	21.3
		2	44.0	8.3	23.7	24.0	43.0	10.0	25.0	22.0	42.5	11.5	27.6	18.4
		3	43.0	9.0	24.0	24.0	42.7	10.0	25.6	21.7	43.0	12.8	25.6	18.6
		4	44.0	10.0	28.0	23.0	41.0	10.0	30.0	19.0	40.6	14.8	28.4	16.2
	Pre-frozen	1F	43.5	7.8	27.0	21.7	47.0	7.6	27.4	22.0	49.5	11.5	32.0	7.0
		2F	46.0	10.3	24.2	19.2	47.0	9.7	20.2	23.1	48.0	10.2	20.3	21.5
		3F	44.2	11.7	25.6	18.7	47.0	8.1	26.5	18.4	44.2	13.4	26.0	16.4
		4F	46.5	10.0	28.0	15.5	45.6	9.8	28.0	16.6	44.2	11.3	26.4	18.2
Mar.	Non-frozen	5	41.8	15.6	22.5	20.0	43.6	7.4	28.6	20.4	41.7	15.7	22.0	20.6
		6	50.0	5.7	24.3	20.0	47.3	4.5	27.6	20.6	51.0	8.0	22.0	19.0
		7	45.7	9.7	23.0	21.6	49.6	9.2	20.0	21.2	49.5	10.6	21.5	18.4
		8	42.8	10.0	28.6	18.6	48.7	7.4	20.6	23.3	50.5	8.9	23.8	16.8
	Pre-frozen	5F	50.0	6.1	21.5	22.4	44.0	6.4	25.7	23.9	50.5	8.9	23.8	16.8
		6F	48.4	7.5	21.5	22.6	40.5	7.0	27.4	25.1	46.0	11.8	26.5	15.7
		7F	40.7	15.2	18.5	25.6	50.0	11.7	23.5	14.8	44.0	13.8	24.7	17.5
		8F	46.8	8.0	22.2	23.0	44.0	6.8	25.7	23.5	45.8	11.4	23.8	19.0

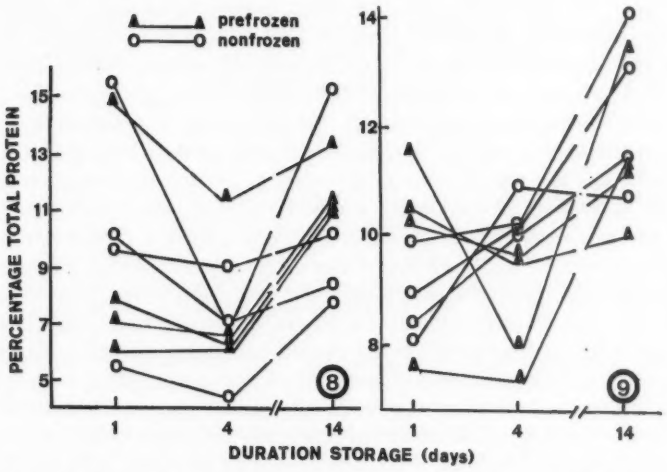

FIG. 8. Percentage of total protein in B bands from nonfrozen and prefrozen fillets with the skin intact graphed as a function of duration of storage at 2 C.
FIG. 9. Percentage of total protein in B bands from nonfrozen and prefrozen skinned fillets graphed as a function of duration of storage at 2 C.

patterns. Even so, that the Pr (X = 3) is equal to 0.047 statistically supports the conclusion that changes in the percentage of protein migrating in the B band are strongly associated with the duration of storage at 2 C for nonfrozen skinned fillets. Although it was apparent that certain variables influence the types of behavior expressed by the B bands, statistical evidence confirms a very strong relationship for all samples between the percentage of protein migrating in the B band and the duration of storage at 2 C.

DISCUSSION

This study has shown that the protein concentration of fish muscle press juice prepared from haddock fillets stored for 2 weeks at 2 C remained remarkably constant between 6 and 8 g/100 ml. Although the fish had undergone gross deterioration after 2 weeks of refrigeration, the concentration of the protein in the fish extract remained essentially unchanged. This supports the conclusion recently reported by other investigators (Stal, 1960) that proteolysis does not occur significantly until after spoilage occurs and is not essential to the spoilage process. Therefore, the protein concentration itself cannot serve as an indicator of freshness.

Qualitative observations made on the densitometry tracings of electropherograms prepared from fish muscle press juice suggested that a number of variables influenced the electrophoretic patterns. Prefreezing the fillets resulted in different patterns than those from the nonfrozen portions only when the skin was removed. Patterns appeared to differ with size, season,

and source of the fillets. But for each fillet, a definite change in the electrophoretic pattern could be seen with respect to storage time. In most instances this change was associated solely with the B band. A quantitative treatment of the data confirmed that the percentage of protein migrating in the B band changed with respect to storage time at 2 C. Significant alterations in the other protein bands did not occur. The statistical relationship between alterations in the B band and storage at 2 C was very strong. Moreover, these changes were predictable as evidenced by strong statistical correlation. However, because so many variables influenced the electrophoretic patterns it was not possible to determine the shelf life of a fillet from a single electrophoretic pattern. Each fillet had a protein pattern characteristic for itself depending on a number of variables, and this pattern changed with respect to storage time. Although such general trends could be followed, it would have been unreasonable to make a judgement on the shelf life of a fillet based on a single electrophoretic pattern influenced by so many variables. Electrophoresis alone seems to offer little promise in determining the shelf life of refrigerated fishery products. Even so, this method has demonstrated that alterations in proteins with specific migratory rates are associated with postmortem changes occurring in tissues of refrigerated fish. Further studies in this area may profit by attempting to resolve the proteins migrating in the B band into several components by disc-gel or immunoelectrophoresis. Such studies might discover that very specific proteins are involved in tissue degradation and careful measurement of the amounts or activities of such proteins could serve to indicate the relative freshness of refrigerated fishery products.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service training grant EH-70-654 from the Bureau of Health Professions Education and Manpower Training.

Gratitude is extended to Dr H. Jean Thiebaux and Mr Leo Hall for their advice and guidance, and to the Department of Microbiology, and the Division of Foods and Drugs at the University of Massachusetts for their cooperation and generosity in providing equipment essential to this study.

REFERENCES

GOULD, E. 1965. Testing the freshness of frozen fish. Fishing News (Book) Ltd., London. 51 p.

HUTTEN, H. 1960. Microelectrophoresis for the estimation of quality of marine fish. Arch. Lebensmittelhyg. 11: 87–90.

LERKE, P., L. FARBER, AND R. ADAM. 1967. Bacteriology of spoilage of fish muscle IV. Role of protein. Appl. Microbiol. 15: 770–776.

MCKEE, J. E., W. L. FAITH, AND H. HEUKELEKICIN. 1963. One hundred problems in environmental health. Jones Comp. Co. and Kirby Lithograph Co., Washington, D.C.

STAL, R. D. 1960. Principles and procedures of statistics with special references to biological sciences. McGraw Hill Book Co. Inc., New York.

STANSBY, M. E. 1958. Problems in determining fish freshness. Food Technol. 12: 260.
1963. Industrial fishery technology. Reinhold Publishing Corp., New York.

TARR, H. L. A. 1954. Microbiological deterioration of fish postmortem; its detection and control. Bacteriol. Rev. 18: 1.

WILSON, A. C. 1967. Enzymatic identification of fish products. Science 157: 82–83.

A Method of Selecting Trout Hybrids (*Salvelinus fontinalis* × *S. namaycush*) for Ability to Retain Swimbladder Gas[1]

J. S. TAIT

Department of Biology
York University, Toronto, Ont.

TAIT, J. S. 1970. A method of selecting trout hybrids (*Salvelinus fontinalis* × *S. namaycush*) for ability to retain swimbladder gas. J. Fish. Res. Bd. Canada 27: 39–45.

A method was developed for selecting hybrid trout for deep-swimming ability, for use in a breeding program to combine in one strain the early-maturing character of brook trout (*Salvelinus fontinalis*) with the deep-swimming ability of lake trout (*S. namaycush*). The method involves testing hybrids in pressure tanks and selecting individuals that, like lake trout, retain most of their swimbladder gas during the test period. For a sample of F_2 hybrids the range of pressures at which the fish floated when anaesthetized was almost entirely between the medians for samples of the two parent species. Successive tests of marked individuals showed good repeatability of flotation measurements. The method is concluded to be reliable for large-scale selection of fish with ability to retain swimbladder gas.

Received March 17, 1969

INTRODUCTION

THIS PAPER DESCRIBES A METHOD of large-scale selection of trout for ability to retain swimbladder gas, reports sample results of selection tests, and assesses the repeatability of measurements made by the selection method. The method is used routinely by the Ontario Department of Lands and Forests in its program to produce, by selective breeding of hybrids of brook trout and lake trout, a trout for planting in the Great Lakes that matures by age II or III with a good chance of reproducing before being killed by sea lampreys or fishermen, and that can live at all depths and take advantage of abundant prey species in both shallow and deep water.

Selection for early maturity is a simple process of saving for brood stock, through several generations, only those hybrids that mature by age III. Selection of fish with ability to live in deep water is attempted by the indirect approach of selecting fish with ability to retain swimbladder gas when prevented from swallowing air. This approach is based on the observation that salmonids that swim deep, such as lake trout, retain swimbladder gas well, whereas shallow-dwelling salmonids, such as brook trout, lose swimbladder gas rapidly (Tait, MS, 1959). The method of testing large numbers of fish for

[1]Contribution No. 68-12 of the Ontario Department of Lands and Forests, Research Branch, Maple, Ont.

Printed in Canada (J1416)

gas loss is essentially the same as the method described earlier (Tait, MS, 1959) for measuring rate of loss of swimbladder gas from individual fish. The amount of gas in the swimbladder is determined indirectly by measuring the flotation pressure, the pressure at which the anaesthetized fish rises from the bottom to the surface of a pressure tank. In the large-scale method, pressure tanks are of 1000–1500 liters capacity instead of 15–50 liters, and procedure differs in that flotation pressures of fish at the beginning of a test period are not measured but are assumed to be the same for all of the fish in the tank.

APPARATUS

The pressure tanks used vary in degree of curvature of the bottom and in total capacity (1000–1500 liters) but otherwise all are alike. A typical tank (Fig. 1) is made of welded steel plate in two parts bolted together with a rubber gasket between them. The lower part is shaped like a shallow bowl 1.5 m in diameter and is supported by four legs of angle iron. The top part is cone-shaped and has a cylinder 0.5 m in diameter welded on below the apex. The part of the cone that extends inside the cylinder is made of molded Plexiglas. At the apex is a 13-cm opening that can be covered by a perforated aluminum flap valve on a stainless steel shaft that passes through a packing gland in the cylinder wall to a control handle. A steel lid, supported by a curved arm, can be lowered onto the top of the cylinder and bolted down by 10 turnbuckles.

An inlet in the lower part of the tank permits either rapid filling of the tank through a gate valve or a small steady flow of water through a needle valve. A second water inlet near the bottom of the cylinder may be used for flushing out the chamber around and above the Plexiglas cone. There are four water outlets: a 10-cm flap valve, held shut by a clamp, at the bottom of the tank for draining the tank; a threaded opening halfway up the side of the cone that may be closed with a plug or fitted with an overflow pipe; a large spout arising from the lower part of the cylinder and leading through a large gate valve to a descending length of pipe; an overflow pipe that extends 1.3 m above the centre of the lid and 3 m above the bottom of the tank and turns down to empty into a funnel.

A small opening in the lid, fitted with a nipple and stopcock, permits connection to a vacuum line, and a stopcock near the bottom of the tank connects to a U-tube mercury manometer. A $\frac{1}{3}$ hp electric motor and a centrifugal pump are mounted beneath the tank. There is a screened intake in the base of the tank for the pump, and the outlet is about halfway up the side of the cone. The intake can be closed by a gate valve, and has a sidearm with a valve and a small funnel through which anaesthetic solution can be admitted. With the intake open, the pump can be used to circulate anaesthetic or to shuffle anaesthetized fish on the bottom of the tank.

There are two Plexiglas windows in the walls of the cone and two in the walls of the cylinder to admit light and permit observation.

PROCEDURE

Because small fish lose gas more rapidly than large ones (Tait, MS, 1959) fish to be tested were sorted into size groups by 1.3-cm intervals below 15 cm fork length and 2.5-cm intervals above 15 cm. Fish of a particular size group were starved for a day and then placed in the tank through the opening at the top of the Plexiglas cone. The number tested at a time ranged from 1200 fish 9 cm long to 50 fish 25 cm long.

For 24 hr the water in the tank overflowed through the outlet halfway up the side, and the fish had an air–water surface at which they could swallow air to add to their swimbladders. It was assumed that during this period of adjustment all of the fish attained neutral buoyancy. At the end of this period the overflow was closed and the water level rose. Before water reached the top of the Plexiglas cone, the circulating pump was run for about 30 sec to remove air pockets in the pumping system. The lid was bolted down tightly, and the water was allowed to overflow through the outlet tube.

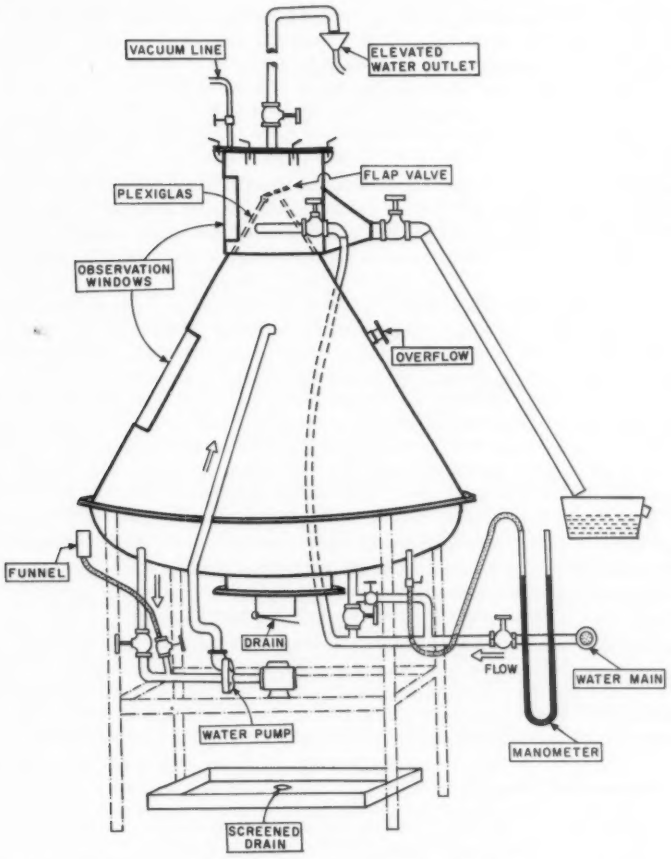

FIG. 1. Flotation tank used in large-scale testing of hybrid trout for ability to retain gas in their swimbladders. Flotation pressure, the pressure at which an anaesthetized fish floats, is measured as the Hg level in the open column of the manometer.

By adjusting the needle valve on the water inlet, the pressure at the bottom of the tank was set at the arbitrarily chosen value of 33 cm Hg above atmospheric pressure. This positive pressure may serve as a stimulus to fill the swimbladder, now reduced in volume because of the pressure increase, and it removed any tendency that the fish might have had to give off gas through the pneumatic duct. Thus any gas that was lost during the test period most probably passed through the swimbladder wall rather than out the pneumatic duct.

For 12 days the fish were left unfed, under pressure of 33 cm Hg above atmospheric, without access to an air surface, in running water at 10 C (±1 summer–winter variation). Bubbles of air that the fish could swallow were not observed in the tank under normal conditions.

At the end of 12 days, the pressure was checked on the manometer and the water was turned off. An anaesthetic solution, 65 g of tricaine methane-sulphonate in water, was poured into the funnel at the circulating pump intake. The pump was started, and with the intake valve almost

closed the valve at the base of the funnel was opened to admit the anaesthetic. Care was taken not to draw in air bubbles. Some rinse water was passed through the funnel, the pump intake valve was opened, and the pump was run for about 5 min to give complete mixing inside the tank. Fifteen to twenty minutes after the anaesthetic was added, the fish were lying on the bottom motionless except for opercular movement. Some water was drained through the spout at the side of the cylinder to bring the water level down to a few centimeters below the lid. The outlet valve above the lid was closed making the tank airtight.

By opening a pinchcock on the vacuum line, the pressure in the tank was gradually reduced until one fish rose slowly from the bottom to the surface, usually head first. The flap valve at the top of the cone was opened, and the fish was allowed to pass up into the cylinder. Pressure was then further reduced to the nearest even number on the right hand column of the manometer, the column open to the air.

For example, if the first fish left the bottom at a reading of 45.7 cm on the right column, the pressure was gradually reduced to 44 cm. Other fish floated up with this reduction, and they were allowed to rise into the cylinder. The fish at the top, having floated at pressures between 44 and 46 cm, were described for convenience as having a flotation pressure of 45. These were the fish with the most buoyant swimbladders at the end of the 12-day test. They were removed from the cylinder through the large gate valve, caught in a tub at the bottom of the descending pipe, and revived in fresh, running water. A jet of water from the valve near the base of the cylinder helped to flush the fish out through the drain spout. The water level in the tank was restored to a few centimeters below the lid, and the pressure was reduced slowly to 2 cm below the level reached before, i.e. to 42 cm in the example. The fish that floated at this pressure were let through the flap valve, removed, and revived. They were described as having a flotation pressure of 43.

This process was continued until all of the fish were floated by 2-cm intervals of pressure or until the limit of the vacuum system was reached, at about 18 cm on the right column. Any fish that could not be floated were removed through the bottom drain.

When anaesthetized, the fish tended to pile up on one another on the bottom. It was necessary two or three times near the beginning of the flotation to stir up the fish by running the circulating pump while at the same time reducing the tank pressure to about 2 cm Hg below the current flotation pressure. When the pump was turned off and atmospheric pressure was restored at the water surface, the fish settled to the bottom, the most buoyant ones tending to be on top of the pile.

The time required between addition of the anaesthetic and removal of the last fish was about 75 min. If more time elapses, the last fish to be removed may not revive.

USE AND REPEATABILITY OF THE METHOD

In a typical test (Fig. 2), the median flotation pressure for lake trout was 46, which if converted to the actual pressure at which the fish floated would be within a centimeter or two of atmospheric pressure. The actual pressure is the atmospheric pressure at the time of flotation minus the difference (left minus right) between the two mercury columns of the manometer. This means that the lake trout lost very little gas during the 12-day period. The median flotation pressure for the brook trout was 20.8, i.e., approximately 23 cm Hg, or 50 cm below atmospheric pressure. The brook trout lost about three-quarters of their swimbladder gas in the 12-day period and 30% of the sample could not be floated with the lowest pressure attainable. The range of the F_2 hybrid sample lay almost entirely between the medians of the parent species, and the F_2 median was intermediate at 32.8.

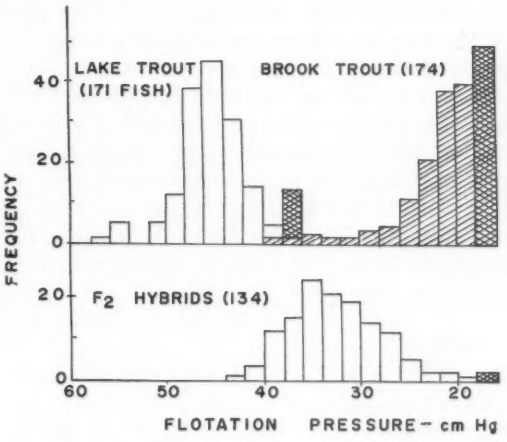

FIG. 2. Frequency distributions of flotation pressures for typical samples of lake trout (Lake Manitou), brook trout (Tarentorous Hatchery stock), and F_2 hybrids (F_2 progeny of Dickson Lake brook trout males and Lake Simcoe lake trout females). The cross-hatched bars represent trout of the three groups that were not floated (lake trout at 36–38 and others at 16–18 cm Hg).

To assess the repeatability of flotation measurements, determinations of flotation pressures of marked fish were compared with later determinations for the same individuals. The flotation measurements shown in Fig. 2 for F_2 hybrids were made on January 16, 1964, and flotations for these fish were determined again on March 9 and June 15, 1964. The average lengths of the fish on the successive test dates were 10.2, 11.1, and 14.6 cm. Similarly, a group of 101 F_3 hybrids were flotation-tested on December 12 and again on December 31, 1960. The flotation pressure determined for each fish on one test date was compared with the flotation pressure for the same fish on each of the other test dates, and the statistical significance of differences between determinations was checked by paired t-test at $P = 0.05$ (Table 1).

TABLE 1. Summary of paired t-tests on typical flotation data for F_3 and F_2 trout hybrids, flotation tested two and three times respectively.

Generation	Date of flotation	No. of pairs in test	Mean flotation pressure (*cm Hg*)	t ($P = 0.05$)[a]
F_3	Dec. 12, 1960	92	32.76±0.55[b]	1.73
	Dec. 31, 1960		32.07±0.52	
F_2	Jan. 16, 1964	116	33.16±0.39	5.75
	Mar. 9, 1964		35.02±0.31	
	Jan. 16	108	33.69±0.38	2.01
	June 15, 1964		34.54±0.41	
	Mar. 9	108	35.24±0.35	1.60
	June 15		34.59±0.42	

[a]A t value of 1.98 is significant at $P = 0.05$ for 100 df.
[b]One standard deviation of the mean.

The two means for the F_3 data were not significantly different. For the F_2 data the January–March means and also the January–June means were significantly different, but the March–June means were not significantly different.

To demonstrate the repeatability of flotation measurements in another way, the data for the 111 F_2 hybrids that survived for three determinations, for example, were treated in the following way. A mean flotation value was calculated from the three determinations for each fish. The deviations of the three determinations from the mean for each fish were tallied to show the frequency distribution of the deviations of the 333 determinations. The data show that 70% of the determinations did not deviate from their means by more than two units (4 cm):

Deviation (±2-cm units)	0	1	2	3	4	5	6	7	8	9	...	15
% of determinations (cumulative)	16	57	70	87	90	95	96	98	98	99	...	100

The tests show that repeatability of the flotation measurements is reasonably good, and that, within certain acceptable limits, the large-scale flotation method is a reliable means of selecting fish with the ability to retain swimbladder gas. The significant difference between the January–March means and that between the January–June means (Table 1) may be partly due to growth. Since swimbladder gas retention in trout has been found to improve with body growth (Tait, MS, 1959) the mean flotation pressures of the F_2 hybrids might be expected to move upward as they did in the tests from January to June.

Since 13% of the deviations from the mean flotation pressures for individuals were greater than three units and 1% were greater than nine units, a single determination for an individual should be used with reservation. If a determination was far below the mean, either the fish may have begun the 12-day period with much less gas than normal in its swimbladder, or it may have lost bubbles of gas through the pneumatic duct during the flotation procedure. In the former instance the fish may have rested on the bottom and did not fill its swimbladder at the surface during the 24-hr adjustment period. The second instance occurs sometimes when fish have been under too long or too heavy anaesthesia. The circular muscles of the duct probably relax and allow bubbles of gas to pass out of the mouth. Such a fish may eventually float but its flotation pressure would be recorded at an erroneously low level.

Deviations above the mean flotation for an individual were not as large as those below. A deviation above the mean could occur if a fish began the 12-day period with more gas than normal in its swimbladder.

In practice, the fish selected for the breeding program are tested a second time to help prevent assigning erroneously high flotation pressures. With this system a fish that does not have a high flotation pressure the first time does not get a second chance. However, any fish having high flotation pressures in two tests is likely to have a high ability to retain swimbladder gas. It remains

to be shown whether hybrids selected for gas-retaining ability actually can live in deep water and to what extent the ability is inherited.

ACKNOWLEDGMENTS

The program for selective breeding of hybrid trout and the design of the flotation tanks were proposed by Dr F. E. J. Fry. It is a pleasure to thank Dr Fry, Dr L. Butler, Mr K. H. Loftus and Mr J. E. Dewar for the advice and support that each has contributed to the research program of which this paper reports a part. I am indebted to Dr Butler, Mr Loftus and Mr A. H. Berst for critical reading of the manuscript.

REFERENCES

TAIT, J. S. MS, 1959. Swimbladder studies in salmonoids relating to problems of depth. Ph.D. Thesis. University of Toronto, Toronto, Ont. 52 p.

Levels of Stable Zn and ^{65}Zn in *Crassostrea virginica* from North Carolina

DOUGLAS A. WOLFE

Bureau of Commercial Fisheries
Radiobiological Laboratory,[1] *Beaufort, North Carolina 28516, USA*

WOLFE, D. A. 1970. Levels of stable Zn and ^{65}Zn in *Crassostrea virginica* from North Carolina. J. Fish. Res. Bd. Canada 27: 47–57.

The concentration of zinc in oysters was highly variable — samples from relatively unpolluted estuaries of North Carolina contained, on the average, 85–245 ppm zinc, based on wet weight. Internal tissues, like adductor muscle and pericardial sac, had zinc levels less than half those of external tissues but zinc was nonetheless distributed uniformly throughout the animal tissues. During 1964–66, North Carolina oysters contained 2–20 pCi ^{65}Zn from fallout per 100 g wet weight. Specific activity of ^{65}Zn in these oysters during 1965–66 was in the range 90–300 pCi/g Zn, and was declining with an apparent half-life of 276 days.

Received September 2, 1969

INTRODUCTION

MEMBERS of the molluscan family Ostreidae have achieved a certain notoriety for their ability to concentrate zinc from sea water (Hiltner and Wichmann, 1919; Bodansky, 1920; Vinogradov, 1953). In recent years, the contamination of estuarine ecosystems by industrial wastes containing heavy metals and by ^{65}Zn from fallout and effluents from nuclear power plants or fuel reprocessing plants has added greater significance to studies on the bioaccumulation of zinc by estuarine organisms (Rice, 1963). Although many measurements of zinc in oyster tissues have been made, the role of zinc in the physiology of the oyster remains unclear. The studies reported herein and in the following paper (Wolfe, 1970) were undertaken to determine background levels of ^{65}Zn distribution and variation of stable zinc, and to clarify the functional significance of the high zinc contents in the American oyster, *Crassostrea virginica* (Gmelin).

Reported values for zinc concentrations in oysters vary over almost two orders of magnitude (Hiltner and Wichmann, 1919; Chipman et al., 1958), probably as a result of seasonal fluctuations (Galtsoff, 1953, 1964; Pringle et al., 1968), species differences, and widely variable zinc concentrations in the water at different sampling locations. In estuarine waters, the zinc concentration may vary with tidal fluctuations, with freshwater runoff, and with the presence of industrial effluents that contain heavy metals. Temperature also could affect the zinc concentration in water by shifting the exchange equilibrium between sediments and water; temperature affects also the bioaccumulation

[1]The Radiobiological Laboratory is jointly supported by the Bureau of Commercial Fisheries and the Atomic Energy Commission.

Printed in Canada (J1583)

of zinc by oysters (Duke et al., 1970; Wolfe and Coburn, 1907). Concentration factors for zinc in oysters appear to be inversely related to the zinc content of the water at concentrations of 10^{-3}–10^{-2} ppm (Preston, 1966a). This relation suggests that the zinc in oysters probably is subject to lesser fluctuations than is the zinc content of the surrounding water.

The oyster's accumulative capacity for zinc and its commercial importance make this organism an especially suitable biological indicator for ^{65}Zn in the environment (Schelske, 1966). The level of ^{65}Zn in oysters is already a significant consideration in the assessment of potential radiation doses to human populations near nuclear power stations in England (Preston, 1966a, b, 1968), and to a much lesser degree, near the mouth of the Columbia River (Foster and Soldat, 1966; Honstead and Hildebrandt, 1967). In estuarine areas not contaminated by nuclear industry, oysters contain residual fallout ^{65}Zn from the intensive nuclear weapons testing of 1961–62, but this activity has diminished considerably since the moratorium on atmospheric testing. The release of very low-level radioactive effluents into estuaries, plus the possibility of a nuclear accident involving a coastal marine environment, make important an accurate estimate of the "background levels" of radioisotopes already in the environment. Such values for ^{65}Zn in oysters from various locations have been published intermittently (Watson et al., 1960, 1963; Murthy et al., 1959; Fitzgerald and Skauen, 1963; Alexander and Rowland, 1966). Included in the present paper are the levels of ^{65}Zn and stable zinc in oysters from the vicinity of Beaufort, North Carolina, at various times during 1964–66.

METHODS

Oysters were captured from subtidal waters (0–2 m) mainly in the North River and Newport River near Beaufort, North Carolina. A few samples also were collected from nearby estuaries along Core Sound and the southern part of Pamlico Sound. Oysters that were used for studies on tissue distribution of zinc were held briefly in the flowing seawater system at the laboratory before being killed. Immediately before being killed, the shells of all animals were scrubbed to minimize contamination of the soft parts by external sand, mud, or epifauna during shucking.

For analysis of ^{65}Zn, the soft parts from as much as a bushel of oysters were dried at 90 C for 24 hr, weighed, then ashed at 450 C for 24 hr. Large samples (40–175 g) of ash were counted on a 4 × 4 inch NaI (T1) scintillation crystal in a low-background shield (Schelske, 1966) for either 80 or 200 min. Gamma spectra were then analyzed as described by Wolfe and Schelske (1969).

Stable zinc was analyzed by atomic absorption spectrophotometry (Perkin-Elmer model 303)[2] or by neutron activation analysis at the Puerto Rico Nuclear Center in Mayaguez, Puerto Rico. For atomic absorption analysis, either wet oyster tissues or ash were dissolved by heating with concentrated HNO_3 until the solution was nearly colorless; the acid was evaporated, and the residue was redissolved in $\frac{1}{4}$ N HCl in deionized distilled water. For neutron activation, ash samples (250–500 mg) were irradiated 4 hr in a neutron flux of 2.5×10^{12} (Phelps, 1966; Lowman et al., 1967). Two months after irradiation, interferences from ^{59}Fe, ^{46}Sc, and ^{60}Co were undetectable, and zinc was analyzed directly with a 4 × 4 inch NaI (T1) crystal and a Nuclear Data 512 Channel Analyzer (Computer Series 130) from comparator standards irradiated with the samples.

[2]Trade names referred to in this publication do not imply endorsement of commercial products.

FALLOUT ^{65}ZN IN OYSTERS

Zinc-65 was measured in two oyster samples collected near the start of 1964 and in several samples during 1965–66. Results were decay-corrected to the date of sample collection and divided by total zinc concentrations to give specific activity (Fig. 1). The initial (1963–64) values for ^{65}Zn in these oysters

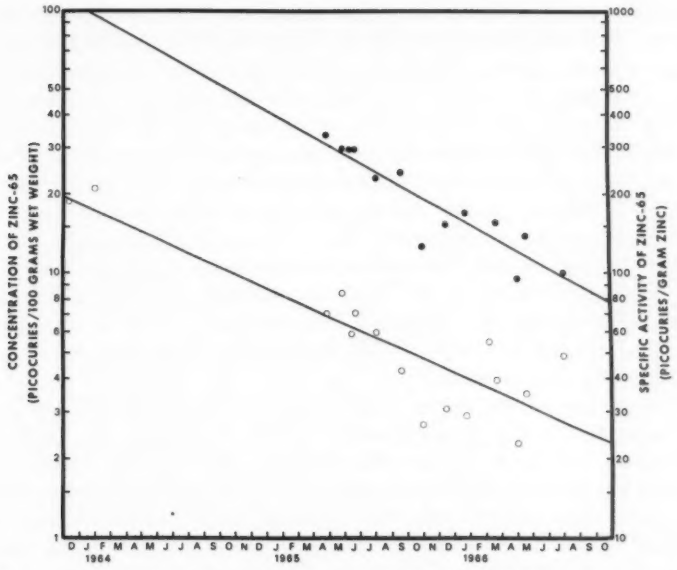

FIG. 1. Zinc-65 from fallout in soft parts of *Crassostrea virginica*: open circles represent absolute activity; solid circles represent specific activity.

corresponded well to those reported for Chesapeake Bay oysters in 1959 (Murthy et al., 1959). The remaining samples (1965–66) generally were slightly less than the value of 91 pCi/kg (converted to wet-weight basis by a factor of 0.2) reported for Chesapeake Bay oysters in 1964–65 (Alexander and Rowland, 1966). The specific activity of ^{65}Zn in Chesapeake Bay oysters, however, was about one-fourth the average value in North Carolina, because of the much higher concentration of stable zinc in oysters from the Chesapeake region.

The absolute and specific activities of ^{65}Zn in oysters showed a regular decline during the period when the samples were collected (Fig. 1). Total zinc concentrations, on the other hand, increased very slightly, but varied considerably, and averaged 241 $\pm$ 74 ppm based on wet weight for the samples shown in Fig. 1.

The semi-logarithmic regression for absolute activity with time had a half-removal time of 347 days (correlation coefficient −0.875), whereas specific

activity, with a half-removal time of 276 days (correlation coefficient −0.918), decreased faster because of the concomitant increase in total zinc. These removal times are the "ecological half-lives" of Held (1960) and Renfro and Osterberg (1969), because the decline of radiozinc in oysters was occurring in the natural environment where simultaneous uptake, loss, and decay of ^{65}Zn could occur. Although very much dependent on the time and location, the ecological half-life is a reasonable means of describing the retention of a radioisotope by a specified organism under environmental conditions. The ecological half-life observed for ^{65}Zn in North Carolina oysters during 1964–66 is longer than the physical half-life of ^{65}Zn (245 days). This relation suggests several conditions that might have characterized ^{65}Zn in the oysters' environment: (1) ^{65}Zn must have been nearly equilibrated with the stable zinc available to the oysters from the environment, i.e., the oysters were presented a relatively static specific activity of ^{65}Zn; (2) the oysters must have been close to equilibrium with respect to the specific activity of ^{65}Zn, so that the biological compartments with long residence times were well labeled; (3) the oyster's ecosystem could conserve ^{65}Zn such that the isotope was retained in a form readily available to oysters; (4) ^{65}Zn was continuously being introduced into the ecosystem during the sampling program; and (5) ^{65}Zn may have been available to oysters at a specific activity higher than that found generally throughout the environment.

The first two of the above conditions are interrelated and are important prerequisites for a long residence time of ^{65}Zn in oysters. In Pacific oysters (*C. gigas*) the biological half-life for ^{65}Zn was estimated at 255 days, which resulted in an effective half-life of only 125 days (Seymour, 1966). Seymour's oysters were well labeled with ^{65}Zn, because they originated from Willapa Bay, Washington, which receives ^{65}Zn from the Hanford Reactor effluent in the Columbia River. The wide disparity between Seymour's effective half-life for ^{65}Zn in oysters and the ecological half-life reported here arises from the simultaneous uptake of ^{65}Zn by oysters in the natural environment. If the environment were totally equilibrated, however, with no new introduction of ^{65}Zn, the ecological half-life in oysters would equal the physical half-life of the isotope, or 245 days. The discrepancy between the estimated ecological half-life and the physical half-life possibly could represent errors in sampling or in estimating radioisotopic and stable Zn, but it suggests also that thorough labeling of long-lived compartments (conditions 1 and 2 above) is not adequate to explain the observed result.

Before the ecological half-life can exceed the physical half-life, the oysters must have a continued supply of ^{65}Zn, as in conditions (3) and (4). Some ^{65}Zn, no doubt, was introduced into the environment from recent fallout during the sampling period — from the stratospheric reservoir remaining from the intensive U.S. and Russian testing of 1961–62, and from Chinese tests concurrent with the sampling. Radiozinc from fallout could also have been continuously available to the oysters, however, from runoff, from tidal exchange with the ocean, or from the sediment substrate beneath the animals. In experimental

ecosystems, sediments were an important reservoir for introduced ^{65}Zn (Duke et al., 1966; Duke, 1967). Previously introduced radioisotopes thus would be retained in the estuarine environment and would be available to organisms for a prolonged time.

The last condition above (number 5) suggests that ^{65}Zn from fallout might not be equilibrated with stable zinc in the environment. Although this possibility is a direct antithesis of conditions (1) and (2), it probably contributes nonetheless to the observed long ecological half-life of ^{65}Zn in oysters. Figure 2 shows the various pathways by which oysters may obtain ^{65}Zn from fallout.

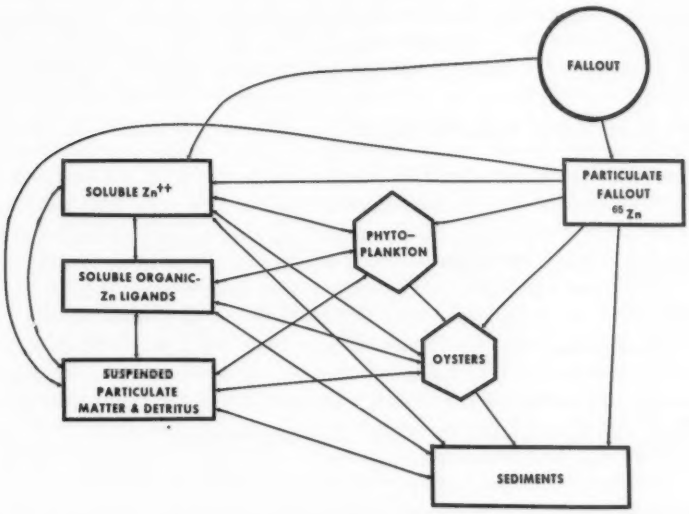

FIG. 2. Hypothetical model showing the different compartments involved in the transfer of ^{65}Zn to oysters in estuarine environments.

If oysters are able to filter particulate fallout material directly from sea water, then this pathway represents a source of ^{65}Zn at very high specific activity. Continued ingestion of particulate fallout could help to provide the ^{65}Zn necessary to prolong the ecological half-life. Since the major reservoirs of stable zinc in the estuarine environment probably are the sediments and suspended particulate matter, any functional line of supply not involving these reservoirs directly will produce a prolonged higher specific activity in oysters. It seems likely that oysters do consume or absorb ^{65}Zn from fallout before it is equilibrated with the stable zinc in the oysters' immediate environment. Because of the complexity of the system, however, no conclusions can be made on the relative significance of the different sources of ^{65}Zn to oysters (Fig. 2), or on the rate of decrease of deposition of ^{65}Zn in fallout. The observed ecological half-life of ^{65}Zn in oysters, however, represents a composite function of the

continuously available, but decreasing supply of the isotope, the biological retention of zinc by the oysters, and the physical decay of the isotope.

STABLE ZINC IN INDIVIDUAL OYSTERS

The concentration of zinc varies widely in oysters from the same location (Table 1). In two groups of 12 oysters analyzed individually for zinc, maximum values were 2.2 and 4.2 times the minimum values. In both groups, the concentration represented by the mean logarithmic value for concentration was nearer the median value than was the arithmetic mean concentration (Table 1). These two groups of oysters suggest that zinc might occur in oyster populations according to a log-normal distribution, as has been observed for trace elements in other organisms (Ting and de Vega, 1969). Atomic absorption analysis of the soft parts from 20 individual oysters agreed reasonably well with results

TABLE 1. Stable zinc in *Crassostrea virginica* from the Newport River (ppm on wet-weight basis).

	Feb. 1966[a]		Jan. 1969	
Expression of concn	Shells (18 samples)	Soft parts (12 samples)	Soft parts[b] (12 samples)	Soft parts[c] (20 samples)
Mean concn ± SD	24.75±9.67	159.4±77.6	245.1±63.5	213.5±83.4
Median value	22.15	136.7	228.7	210.5
Concn at mean log value	23.61	142.9	237.3	194.0

[a]Animals were resident in the laboratory seawater system for 4 months before analysis by atomic absorption.
[b]Analyzed by neutron activation, Puerto Rico Nuclear Center.
[c]Analyzed by atomic absorption.

of neutron activation analysis of 12 oysters from the same sample (Table 1). Two of the oysters in this group, however, produced unusually low values (50 and 72 ppm) compared to the range of the remaining 18 (132–368 ppm), and the zinc concentrations appeared to be distributed normally among these 20 oysters. Because of very wide individual variation among oysters, therefore, it appears that much larger samples must be analyzed by a single technique before the distribution of zinc concentrations can be described conclusively as normal or log-normal.

Soft parts of oysters showed on the average about six times the zinc concentration of shells (Table 1). Since the soft parts accounted for only 19.5 ± 4.1% of the total live weight of the oysters, however, the shells contained nearly 45% of the total zinc in this group of oysters. The concentration of zinc in "living" oyster shell probably depends on the amount of zinc available to the organism, or even more likely, on the available zinc/calcium ratio. Once deposited in shells, zinc is probably no longer available to other organisms. Nonliving geological deposits of oyster shell contain less zinc (2–9 ppm), however, than is reported here for fresh samples (Tressler, 1923; Smith and

Wright, 1962). Zinc may be released from the shell matrix very slowly, so that shells represent a significantly large reservoir of zinc with a slow turnover in the environment.

Newport River water was analyzed for zinc at monthly intervals during 1967–68 at stations near the laboratory and near the source of the oysters in Table 1 (Cross et al., 1970). Dithizone extraction of whole, untreated river water produced median values of 0.55 and 0.75 μg/liter (ranges for 10 samples: <0.1–1.4 μg/liter and <0.1–1.3 μg/liter, respectively). If the water was treated with acid before extraction, results were higher by a factor of 1.1–2.4. Calculation of average concentration factors for zinc in oysters is complicated, therefore, because we do not know what fraction of total zinc in the water is available to oysters, either directly or through the food chain. In addition, the trace element content of oysters appears to be related not only to the concentration of the specific element, but also to the salinity of environmental water (Rucker and Valentine, 1961). Nonetheless, if 1.0 μg/liter is accepted as a representative value for zinc in Newport River water, then concentration factors in oysters (calculated from data in Tables 1–3) are on the order of 1 or 2 $\times$ 10^5 for undrained soft tissues, and as much as 4 $\times$ 10^5 for drained meats.

Zinc concentration in oysters varies widely, depending upon the source and time of the sample (Pringle et al., 1968). Unfortunately, as in the present study, investigators have not yet examined the long-term variability of elemental concentrations in environmental water in conjunction with concentrations in shellfish, and the validity of concentration factors (e.g., for zinc in oysters) is not well understood. Where investigators have measured zinc in the water concurrently with the collection of oyster samples, the data suggest that zinc concentration factors in oysters are inversely related to the zinc content of the water (Chipman et al., 1958; Preston, 1966a). Thus, oysters may become "saturated" with zinc at some level, where further increases in environmental zinc concentrations would cause less and less increase in the zinc content of the organism. Another complicating factor is the lag time between changes in environmental zinc concentration and the appropriate response in the zinc content of oysters. The zinc content of estuarine water is probably subject to more rapid fluctuations than is that of oysters, and therefore the concentration factors measured in oysters could be subject to wide variation. Also, zinc concentrations of natural estuarine waters are frequently at such a low level that large relative errors can be encountered in analysis; this problem can produce large errors in estimates of concentration factors. Because of the uncertainties inherent in the determination of concentration factors and specific activity of isotopes in the environment, caution must be exercised in the application of both these factors to the prediction of potential levels of radioisotopes like ^{65}Zn in selected organisms in contaminated environments.

TISSUE DISTRIBUTION OF ZINC IN OYSTERS

Fifteen oysters collected in early April 1965 from the northwest shore of Pivers Island in the Newport River estuary were dissected; after the pooled tissue fractions were homogenized

with 6 or 8 (muscle) volumes of Tris–HCl buffer (0.05 M, pH 8.0), aliquots were digested in HNO_3 and analyzed by atomic absorption for zinc (Table 2). Pallial fluid was collected immediately upon opening the shell of each oyster. All other fluids that seeped from the tissues during dissection were combined as extracellular fluid. In this dissection, the greenish-brown digestive gland and gut contents were separated as well as possible from the remainder of the visceral mass.

TABLE 2. Percentages of total wet weight and total zinc contributed by different tissues of *Crassostrea virginica*, Newport River, April 1965.

Tissue	% of total wet wt	ppm zinc	% of total zinc
Mantle	16.5	135	26.1
Gills	7.6	182	16.1
Labial palps	3.6	123	5.2
Muscle	7.2	79	6.7
Digestive gland	8.0	260	24.5
Remainder	9.3	175	19.1
Extracellular fluid	27.5	6.5	2.1
Pallial fluid	20.3	1.2	0.3
Total	100.0	85.4	100.1
Less pallial fluid	79.7	106.7	99.8
Less extracellular fluid	52.2	162.7	97.7

In November 1966, tissue distribution of zinc was reinvestigated with 16 oysters collected from North River (Table 3). The dissection differed from the first in that the pericardial sac and rectum were separated from the visceral mass, and the gonadal tissue was then dissected from the remaining digestive

TABLE 3. Percentages of total wet weight and total zinc contributed by different tissues of *Crassostrea virginica*, North River, November 1966.

Tissue	% of total wet wt	ppm zinc	% of total zinc
Mantle	14.8	450.6	30.6
Gills	8.8	508.1	20.4
Labial palps	3.4	501.2	7.7
Muscle	6.8	126.1	4.0
Digestive gland	12.0	491.8	27.1
Rectum	0.4	531.0	1.1
Pericardial sac	0.3	223.9	0.3
Gonad	2.7	435.9	5.3
Extracellular fluid	28.1	18.8	2.4
Pallial fluid	22.7	11.4	1.1
Total	100.0	218.5	100.0
Less pallial fluid	77.3	279.3	98.9
Less extracellular fluid	49.2	427.8	96.5

tissue. The relative zinc content of the digestive gland tissue is therefore reflected in Table 2, whereas that in gonadal tissue is shown in Table 3. Neither the percentage of total zinc nor the percentage of total tissue weight for these two fractions (digestive gland – remainder in Table 2; digestive gland – gonad in Table 3) can be considered accurate, however, because the gonadal tissue was so intimately associated with the visceral mass in the oysters. It is obvious, nonetheless, that the digestive gland consistently had a higher zinc content than gonadal tissue.

Zinc is distributed fairly uniformly throughout oyster tissues, but tissues with exposed surfaces (gills, mantle, labial palps, gonad, and digestive gland) contain more zinc than do internal tissues, such as muscle and pericardial sac (Tables 2 and 3). Although the dissections reported here were perhaps more thorough than were those previously published (Bodansky, 1920; Koga, 1934; Galtsoff, 1964; Price and Lewis, 1966; Ikuta, 1968), results were similar, and no conclusions can be drawn on the physiological role of zinc in any tissue. In the freshwater mussel *Anodonta*, however, zinc and manganese are highly concentrated in calcareous tissue, which is a somewhat diffuse tissue with a very high calcium content, apparently with a storage function related to shell formation. Mantle and gill tissues of *Anodonta* contained less zinc or manganese than did the separated calcareous tissue (Harrison, 1969). No references to calcareous tissue in Ostreidae can be found. Perhaps "calcareous cells" are so widely dispersed throughout the organism that minerals appear evenly distributed. The accumulation of zinc and other trace metals may be partly a coincidental result of poor discrimination by the biological mechanism for calcium uptake and shell deposition. This possibility is consistent with the observed seasonality of trace-element concentration (Galtsoff, 1953, 1964), where contents of manganese, iron, copper, and zinc were higher during warm months when shell deposition is greatest.

ACKNOWLEDGMENTS

I appreciate the technical assistance of Jeraldine Brooks, Jo-Ann Lewis, and Twyla Miner. I also thank F. A. Cross, J. P. Baptist, and T. R. Rice of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, North Carolina, for critical review of the manuscript. The research was supported by U.S. Atomic Energy Contract AT(49-7)-5.

REFERENCES

ALEXANDER, G. V., AND R. H. ROWLAND. 1966. Estimation of zinc-65 background levels for marine coastal waters. Nature 210: 155–157.

BODANSKY, M. 1920. Biochemical studies on marine organisms. II. The occurrence of zinc. J. Biol. Chem. 44: 399–407.

CHIPMAN, W. A., T. R. RICE, AND T. J. PRICE. 1958. Uptake and accumulation of radioactive zinc by marine plankton, fish, and shellfish. U.S. Fish Wildlife Serv. Fish. Bull. 58: 279–292.

CROSS, F. A., T. W. DUKE, AND J. N. WILLIS. 1970. Biogeochemistry of trace elements in a coastal plain estuary. Fe, Mn, and Zn in sediments, water and polychaete worms from Newport River, N.C. Annu. Rep. Bur. Commer. Fish. June 30, 1969. U.S. Fish. Wildlife Serv. Circ. (In press.)

DUKE, T. W. 1967. Possible routes of zinc 65 from an experimental estuarine environment to man. J. Water Pollut. Contr. Fed. 39: 536–542.

DUKE, T. W., J. N. WILLIS, AND T. J. PRICE. 1966. Cycling of trace elements in the estuarine environment. I. Movement and distribution of zinc 65 and stable zinc in experimental ponds. Chesapeake Sci. 7: 1–10.

DUKE, T., J. WILLIS, T. PRICE, AND K. FISCHLER. 1969. Influence of environmental factors on the concentrations of ^{65}Zn by an experimental community, p. 355–362. *In* D. J. Nelson and F. C. Evans [ed.] Radioecology, Proc. 2nd Nat. Symp., USAEC-CONF-670503.

FITZGERALD, B. W., AND D. M. SKAUEN. 1963. Zinc-65 in oysters in Fishers Island Sound and its estuaries, p. 159–162. *In* V. Schultz and A. W. Klement [ed.] Radioecology. Reinhold, New York.

FOSTER, R. F., AND J. K. SOLDAT. 1966. Evaluation of the exposure resulting from the disposal of radioactive wastes into the Columbia River, p. 683–696. *In* Disposal of radioactive wastes into seas, oceans and surface waters. IAEA, Vienna.

GALTSOFF, P. S. 1953. Accumulation of manganese, iron, copper, and zinc in the body of American oyster, *Crassostrea (Ostrea) virginica*. Anat. Rec. 117: 601–602.
1964. The American oyster, *Crassostrea virginica* Gmelin. U.S. Fish Wildlife Serv. Fish. Bull. 64: iii + 480 p.

HARRISON, F. L. 1969. Accumulation and distribution of ^{54}Mn and ^{65}Zn in freshwater clams, p. 198–220. *In* D. J. Nelson and F. C. Evans [ed.] Radioecology, Proc. 2nd Nat. Symp., USAEC-CONF-670503.

HELD, E. E. 1960. Land crabs and fission products at Eniwetok Atoll. Pac. Sci. 14: 18–27.

HILTNER, R. S., AND H. J. WICHMANN. 1919. Zinc in oysters. J. Biol. Chem. 38: 205–221.

HONSTEAD, J. F., AND P. W. HILDEBRANDT. 1967. Uptake and retention of zinc-65 from certain foods. Health Phys. 13: 649–652.

IKUTA, K. 1968. Studies on accumulation of heavy metals in aquatic organisms. III. On accumulation of copper and zinc in the parts of oysters. Bull. Jap. Soc. Sci. Fish. 34: 117–122.

KOGA, A. 1934. Repartition du zinc dans les organes des animaux. Keio. J. Med. 5: 97. (Cited by A. P. Vinogradov (1953), p. 358.)

LOWMAN, F. G., D. K. PHELPS, R. Y. TING, J. H. MARTIN, D. J. SWIFT, AND R. MCCLIN ESCALERA. 1967. Puerto Rico Nuclear Center marine biology program, progress summary report No. 5 (June 1966–June 1967). PRNC No. 104. 166 p.

MURTHY, G. K., A. S. GOLDIN, AND J. E. CAMPBELL. 1959. Zinc-65 in foods. Science 130: 1255–1256.

PHELPS, D. K. 1966. Partitioning of the stable elements Fe, Zn, Sc, and Sm within a benthic community, Añasco Bay, Puerto Rico, p. 721–734. *In* B. Åberg and F. P. Hungate [ed.] Radioecological concentration processes. Pergamon Press, Oxford.

PRESTON, A. 1966a. Concentration of ^{65}Zn in the flesh of oysters related to the discharge of coaling pond effluent from the C.E.G.B. Nuclear Power Station at Bradwell-on-Sea, Essex, p. 995–1004. *In* B. Åberg and F. P. Hungate [ed.] Radioecological concentration processes. Pergamon Press, Oxford.
1966b. Site evaluations and the discharge of aqueous radioactive wastes from civil nuclear power stations in England and Wales, p. 725–737. *In* Disposal of radioactive wastes into seas, oceans and surface waters. IAEA, Vienna.
1968. The control of radioactive pollution in a North Sea oyster fishery. Helgoländer Wiss. Meeresunters. 17: 269–279.

PRICE, T. J., AND C. W. LEWIS. 1966. Accumulation of radionuclides by invertebrates, p. 21–22. *In* Annual report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1965. U.S. Fish Wildlife Serv. Circ. 244.

PRINGLE, B. H., D. E. HISSONG, E. L. KATZ, AND S. T. MULAWKA. 1968. Trace metal accumulation by estuarine mollusks. J. Sanit. Eng. Div., Proc. Amer. Soc. Civil Eng. 94: 455–475.

RENFRO, W. C., AND C. OSTERBERG. 1969. Radiozinc decline in starry flounders after temporary shutdown of Hanford reactors, p. 372–379. *In* D. J. Nelson and F. C. Evans [ed]. Radioecology, Proc. 2nd Nat. Symp., USAEC-CONF-670503.

RICE, T. R. 1963. Review of zinc in ecology, p. 619–631. *In* V. Schultz and A. W. Klement [ed.] Radioecology. Reinhold, New York.

RUCKER, J. B., AND J. W. VALENTINE. 1961. Salinity response of trace element concentration in *Crassostrea virginica*. Nature 190: 1099–1100.

SCHELSKE, C. L. 1966. The fate of fallout radioactivity in an estuary. Amer. Biol. Teacher 28: 373–380.

SEYMOUR, A. H. 1966. Accumulation and loss of zinc-65 by oysters in a natural environment, p. 605–619. *In* Disposal of radioactive wastes into seas, oceans and surface waters. IAEA, Vienna.

SMITH, R. A., AND E. R. WRIGHT. 1962. Elemental composition of oyster shell. Tex. J. Sci. 14: 222–224.

TING, R. Y., AND V. R. DE VEGA. 1969. The nature of the distribution of trace elements in longnose anchovy (*Anchoa lamprotaenia* Hildebrand), Atlantic thread herring (*Opisthonema oglinum* LaSueur), and alga (*Udotea flabellum* Lamouroux), p. 527–534. *In* D. J. Nelson and F. C. Evans [ed.] Radioecology, Proc. 2nd Nat. Symp., USAEC-CONF-670503.

TRESSLER, D. K. 1923. Marine products of commerce. Chemical Catalogue Co., New York. 762 p.

VINOGRADOV, A. P. 1953. The elementary chemical composition of marine organisms. Sears Found. Mar. Res., Yale Univ., Mem. 2. 647 p.

WATSON, D. G., J. J. DAVIS, AND W. C. HANSON. 1960. Zinc-65 in marine organisms along the Oregon and Washington coasts. Science 133: 1826–1828.

1963. Interspecies differences in accumulation of gamma emitters by marine organisms near the Columbia River mouth. Limnol. Oceanogr. 8: 305–309.

WOLFE, D. A. 1970. Zinc enzymes in *Crassostrea virginica*. J. Fish. Res. Bd. Canada 27: 59–69.

WOLFE, D. A., AND C. B. COBURN, JR. 1970. Influence of salinity and temperature on the accumulation of cesium-137 by an estuarine clam under laboratory conditions. Health Phys. (In press.)

WOLFE, D. A., AND C. L. SCHELSKE. 1969. Accumulation of fallout radioisotopes by bivalve molluscs from the lower Trent and Neuse Rivers, p. 493–504. *In* D. J. Nelson and F. C. Evans [ed]. Radioecology, Proc. 2nd Nat. Symp., USAEC-CONF-670503.

Zinc Enzymes in *Crassostrea virginica*

DOUGLAS A. WOLFE

Bureau of Commercial Fisheries
Radiobiological Laboratory,[1] *Beaufort, North Carolina 28516, USA*

WOLFE, D. A. 1970. Zinc enzymes in *Crassostrea virginica*. J. Fish. Res. Bd. Canada 27: 59–69.

Nearly all the zinc in oysters is bound, either to soluble high-molecular weight proteins or to structural cellular components such as cell membranes. Oyster alkaline phosphatase is a zinc metalloenzyme, as indicated by in vitro inhibition studies with various metal-binding agents. Dialysis of soluble tissue extracts at *p*H 7–9 removes up to 96% of the total zinc without effect on alkaline phosphatase. If alkaline phosphatase is considered representative of the metabolic functions of zinc in oysters, most of the zinc accumulated by oysters must be superfluous to the animal's requirements.

Received September 2, 1969

INTRODUCTION

ZINC is found in biological systems as an integral component of certain enzymes and as a loosely bound component of several other enzymes (Vallee, 1962). Despite extensive studies (Wolfe, 1970) on the gross accumulation and distribution of zinc in oysters, however, very little is known about the extent and nature of zinc–protein interaction in this animal. On the basis of a single dialysis experiment, Bodansky (1920) suggested that zinc was at least partly combined with protein in oysters, and that the zinc–protein complex may dissociate during dialysis. According to a recent computation on the absolute zinc requirement of biological tissues, oysters accumulate zinc to a level several orders of magnitude greater than that required for essential metabolic functions (Pequegnat et al., 1969). Korringa (1952) hypothesized that "During feeding the oyster probably cannot avoid collecting and ingesting those ions which adhere firmly to the mucous feeding sheets. Collecting these ions (heavy metals) is less of a problem to the oyster than getting rid of them." Oysters undoubtedly accumulate trace metals like zinc also during ingestion of food and from coincidental ingestion of suspended sediment. Particulate matter may also be ingested directly into cells of the mantle epithelium of Pelecypoda by pynocytosis (Bevelander and Nakahara, 1966; Nakahara and Bevelander, 1967). In Crustacea, adsorption-exchange probably accounts for much of the zinc accumulation, since autoradiographs showed that zinc-65 was localized on the surfaces of the integument and underlying soft tissue after 14 days of accumulation (Cross et al., 1968). A similar phenomenon seems likely to occur on the extensive exposed surfaces of the gills, mantle, and labial palps of

[1]The Radiobiological Laboratory is jointly supported by the Bureau of Commercial Fisheries and the Atomic Energy Commission.

Printed in Canada (J1584)

oysters, all of which contain higher zinc concentrations than do internal tissues (Wolfe, 1970).

In other biological systems, zinc is an integral component of carbonic anhydrase, alcohol dehydrogenase, glutamate dehydrogenase, lactic dehydrogenase, carboxy-peptidase, alkaline phosphatase, and probably also other pyridine-nucleotide-dependent enzymes (Vallee, 1962). In this paper are reported in vitro studies on zinc–protein associations in homogenates and extracts of oyster tissues, measurements of supposed zinc enzymes in oyster extracts, and experiments on the inhibition of alkaline phosphatase by metal-binding agents.

METHODS

Oysters (*Crassostrea virginica*) were collected from the North River estuary near Beaufort and maintained in the laboratory's flowing seawater system until used. Zinc and other trace metals were analyzed by atomic absorption spectrophotometry (Perkin-Elmer Model 303).[2] Biological tissues were wet-ashed with HNO_3 as described by Wolfe (1970). Glassware was cleaned before use by soaking in sulfuric acid-dichromate solution followed by repeated rinsing with distilled water, $\frac{1}{4}$ N HCl, and deionized distilled water. Whenever possible, solutions were kept in polyethylene containers. All buffers used were in deionized distilled water. Protein was determined by the empirical method of Layne (1957) from the ultraviolet absorption at 260 and 280 mμ. Nitrogen was determined by a modified micro-Kjeldahl procedure in an all-glass distillation apparatus, as described by Hiller et al. (1948).

Enzyme analysis — All spectrophotometric enzyme assays were performed with a Beckman DU spectrophotometer equipped with a thermostatically controlled cell housing (25 C) and a photomultiplier attachment. Dehydrogenases were assayed by the spectrophotometric measurement of NAD reduction or NADH oxidation at 340 mμ in the presence of the appropriate substrate, as follows: pyruvate for lactic dehydrogenase; ethanol for alcohol dehydrogenase; glutamate for glutamic dehydrogenase; and oxaloacetate for malic dehydrogenase. Substrates, NAD, and NADH were obtained from Calbiochem Corp. Carboxy-peptidase activity was tested by the general method of Folk and Schirmer (1963) with hippuryl-L-phenylalanine, hippuryl-L-arginine, or hippuryl-L-lysine (Sigma) as substrate. Alkaline phosphatase was determined spectrophotometrically at 420 mμ with *p*-nitrophenylphosphate (PNPP, as either the disodium or di(cyclohexylammonium) salt, from Calbiochem) as substrate (Garen and Levinthal, 1960). Carbonic anhydrase was estimated in terms of the time required to change the *p*H, measured with a *p*H meter, of 3 ml of 0.02 M veronal buffer solution mixed with 2 ml CO_2-saturated deionized water at 0 C from 8.1 to 6.3 (Wilbur and Anderson, 1948). The time without enzyme is recorded as T_0; and with enzyme, T (Worthington, Biochemical Corporation, 1968).

Metal-binding agents — Reagent grade commercial chemicals were used without further purification. With the exception of ammonium pyrrolidino-dithiocarbamate (K & K Laboratories), all were Fischer Certified Reagents.

ZINC–PROTEIN ASSOCIATION IN OYSTERS

Centrifugal fractionation — Aliquots of tissue homogenates from 15 oysters (Wolfe, 1970, Table 2) were centrifuged (Sorvall Superspeed Model RC-2, head type SS-34) 10 min at 2500 rpm (750 × g), and the supernatant solutions were decanted and recentrifuged 20 min at 12,000 rpm (17,000 × g). The precipitates from the centrifugations and the remaining supernatant solution were digested separately with HNO_3 and the residues were dissolved in 0.25 N HCl

[2]Trade names referred to in this publication do not imply endorsement of commercial products.

and analyzed for zinc. Results are compared with the amount of zinc in unfractionated homogenate in Table 1.

Zinc occurred in approximately the same proportions in the centrifugal fractions, regardless of the tissue (Table 1). In all (except for pallial and extracellular fluids) zinc remained largely with the cellular debris (750 × *g* PPT) and the supernatant fractions. Although the amount of material precipitated at 17,000 × *g* was very small, appreciable quantities of zinc were in this fraction, especially for muscle tissue. Unfortunately, the fraction was not characterized nor was the concentration of zinc per unit weight of precipitate determined. Only 30–48% of the total zinc in oyster tissues remained in the supernatant after centrifugation at 17,000 × *g* for 20 min, whereas in the extracellular fluid and pallial fluid, about 90% occurred in the soluble fraction.

Gel filtration of oyster extracts — Extracellular fluid from 12 oysters was centrifuged at 2000 rpm (480 × *g*) for 12 min to remove small bits of tissue. One ml of the resulting supernatant solution, which contained about 6.3 mg protein and 6.0 μg zinc, was eluted from a column of Sephadex G-100 with 0.05 M Tris–HCl buffer, *p*H 8.0. Zinc and protein were measured in the 5-ml fractions collected (Fig. 1).

Only two major fractions with protein absorption were obtained; the lesser of them consisted of small molecules, probably amino acids and peptides, because sodium was eluted from the column in the same region (fraction numbers 15–17). Zinc was associated almost exclusively (96.3%) with the large first protein fraction (fraction numbers 4–8). The absence of further protein fractionation on Sephadex G-100 suggests that the protein molecules in extracellular fluid of oysters are all very similar, with molecular weights greater than 100,000. Similar elution patterns for protein have been obtained from oyster blood on Sephadex G-200 (Gaumer, 1966).

Similar experiments were conducted with supernatant solutions from oyster homogenates. When whole soft parts of oysters were homogenized and the cellular debris was sedimented by centrifugation at 10,000 rpm (12,000 × *g*) for 10–30 min, the supernatant contained, on the average, 86 μg/ml zinc, 4.04 mg/ml N, and about 40 mg/ml protein. Gel filtration of this supernatant on a polyacrylamide resin (Bio-Gel P300) with an exclusion limit of 300,000 mol wt produced results very much like those obtained with extracellular fluid (Fig. 2). Again, only two protein-absorbing fractions were separated, and essentially all (98.3%) of the zinc was eluted before the second peak in UV absorption. This time, however, only about 55% of the total zinc actually occurred within the major protein fraction (fraction numbers 16–36); about 39% was eluted with the intervening fractions between numbers 37 and 74. This distribution of zinc implies that the low UV absorption in fractions 37–74 arises from proteins of less than 300,000 mol wt, even though no discrete protein fraction appeared. Alkaline phosphatase also was assayed in these column fractions, and is shown in Fig. 2 as μmoles *p*-nitrophenyl phosphate hydrolyzed per hour per fraction. Alkaline phosphatase occurred only in fractions 23–36, where it paralleled the protein absorption and zinc concen-

TABLE 1. Distribution of zinc in centrifugal fractions of oyster homogenates.

Tissue	Zinc, μg/ml of homogenate					% of total in fraction		
	750 × *g* PPT	17,000 × *g* PPT	Supernate	Sum	Unfractionated homogenate	750 × *g* PPT	17,000 × *g* PPT	Supernate
Mantle	8.3	1.06	8.5	17.86	19.3	46.5	5.9	47.6
Gills	15.8	0.50	9.0	25.3	26.0	62.5	2.0	35.5
Labial palps	8.3	0.89	7.65	16.84	17.6	49.3	5.3	45.4
Muscle	5.0	0.80	3.45	9.25	8.8	54.1	8.6	37.3
Digestive gland	26.0	1.11	11.8	38.91	37.1	66.8	2.9	30.3
Remainder	14.2	1.15	8.3	23.65	25.0	60.0	4.9	35.1
Extracellular fluid	[a]	0.57	4.3	4.87	6.5	[a]	4.2	95.8
Pallial fluid	[a]	0.05	1.15	1.20	1.2	[a]	11.7	88.3

[a]Extracellular and pallial fluids were not centrifuged at 750 × *g*.

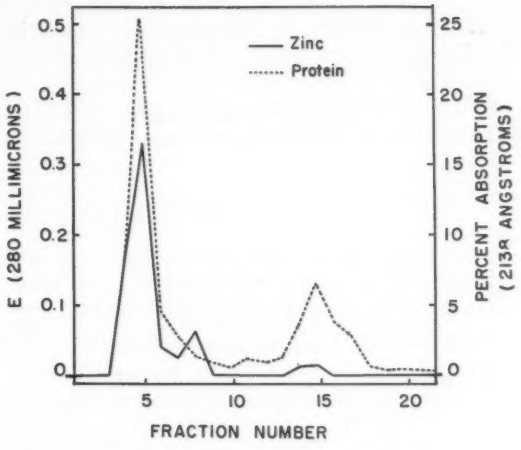

FIG. 1. Gel filtration of extracellular fluid from oysters on Sephadex G-100.

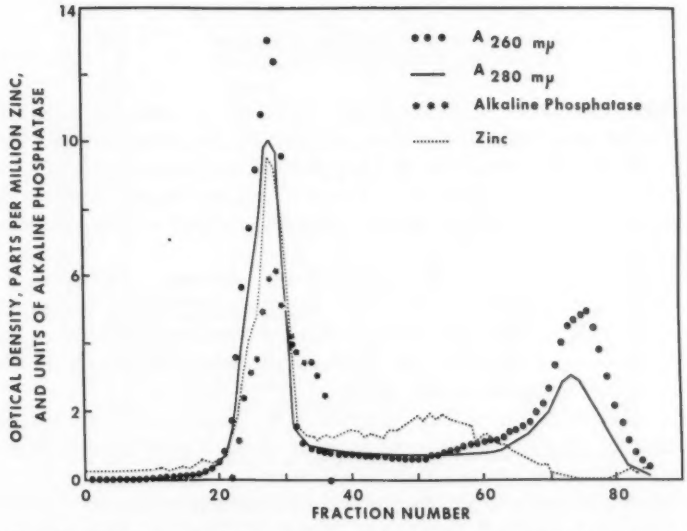

FIG. 2. Gel filtration of the supernatant fraction from an oyster homogenate on Bio-Gel P-300, showing the distribution of protein, zinc, and alkaline phosphatase.

tration at the peak activity, but tapered off more gradually (fraction numbers 31–36) than did either zinc or protein.

SUPPOSED ZINC ENZYMES IN OYSTER HOMOGENATES AND EXTRACTS

The soft parts of 10 oysters were homogenized individually; the homogenates were centrifuged, and the supernatant solutions were assayed for

the enzymatic activities shown in Table 2. Only three of the seven enzymes tested were detected in the extracts of whole oysters. The procedures and results obtained are discussed separately for each enzyme.

TABLE 2. Presumed zinc enzymes in oyster homogenates.

Enzyme	Concn (*units/20 mg extracted oyster*)
Carbonic anhydrase	0.25±0.1[a]
Alkaline phosphatase	1.78±0.45[b]
Malic dehydrogenase	20.4±4.6[c]
Carboxy-peptidase	Not detectable
Alcohol dehydrogenase	" "
Glutamic dehydrogenase	" "
Lactic dehydrogenase	" "

[a] $\frac{T_0 - T}{T}$, for *p*H change from 8.1 to 6.3 (Worthington, 1968).

[b] Micromoles PNPP hydrolyzed per hour.
[c] Micromoles NADH oxidized per hour.

Carbonic anhydrase — Considerable variation was encountered in extracts of different oysters and in duplicate analysis of the same oyster. The value on Table 2 might, therefore, best be regarded as a confirmation of the qualitative presence of carbonic anhydrase in oyster extracts, a presence that was reported previously at approximately the same concentration and with similar variability (Freeman and Wilbur, 1948).

Alkaline phosphatase — The assayed activity was proportional to the amount of protein added to the assay mixture in the concentration range reported in Table 2. The enzyme was stable for several days when stored at 1–5 C. Results were similar whether the substrate was presented as the disodium or the di(cyclohexylammonium) salt.

Malic dehydrogenase — The activity was proportional to protein concentration, and the enzyme was stable for at least 3 days when extracts were stored at refrigerator temperature (2–4 C).

Carboxy-peptidase — The assay was tested satisfactorily on soluble supernatant from homogenized beef liver, obtained from a local supermarket. Oystet preparations, however, gave negative results, even when a portion of the entire homogenate was added to the assay mixture. No change in absorbance could be detected at 254 mμ with any of the three hippuryl-peptides as substrate.

Alcohol, glutamic, and lactic dehydrogenases — The assays performed satisfactorily on beef liver (ADH, GLDH), commercial baker's yeast (ADH), or pinfish (*Lagodon rhomboides*) serum (LDH), but all failed to give positive tests with oyster preparations at any concentration. These dehydrogenases also failed to show when tested with the appropriate substrate after

electrophoresis of oyster extracts on slabs of starch gel (D. W. Engel and D. A. Wolfe, unpublished observations). The absence of alcohol and lactic dehydrogenases is consistent with previous observations on the low formation of lactic acid in molluscs during anaerobiosis and suggests that alternative pathways operate for the disposition of pyruvate and acetate in oysters (Martin, 1961; Bennett and Nakada, 1968). No references to glutamate dehydrogenase in oysters have been located.

METAL REQUIREMENTS OF ALKALINE PHOSPHATASE

Several nonspecific metal-binding agents were mixed in various proportions with the assay mixture for alkaline phosphatase, just before addition of the oyster extract. The ability of any of the compounds tested to inhibit alkaline phosphatase (Fig. 3) demonstrates the dependence of oyster alkaline phos-

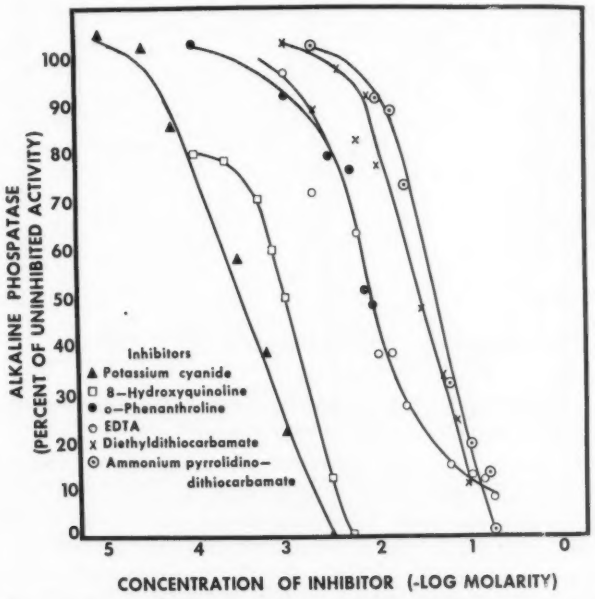

FIG. 3. Instantaneous inhibition of oyster alkaline phosphatase by several different metal-complexing agents.

phatase on a trace metal. Addition of Cu, Cd, Mn, or Zn directly to the assay mixtures containing 10^{-3} M sodium cyanide was shown previously to reverse partially the enzyme inhibition at metal:cyanide molar ratios of 0.2–5.0 (Wolfe and Miner, 1967). Copper was most effective at reversing instantaneous inhibition of alkaline phosphatase by sodium cyanide; zinc was the least. Since the metal and the cyanide were mixed before addition of the enzyme,

however, the order of effectiveness of reversal probably indicated the relative stabilities of the complexes between cyanide and the respective added metal ions instead of the relative effectiveness of promoting alkaline phosphatase activity.

When extracts of oyster homogenates are dialyzed against successive daily changes of 0.05 M Tris–HCl buffer, *p*H 8.0, zinc is removed from the extract (Fig. 4). About 95% of the original zinc content is removed by day 14, but if dialysis is continued 14 days longer, little additional zinc is removed. Alkaline phosphatase activity is not significantly affected by this loss of zinc from the preparation. The slight decrease in alkaline phosphatase (Fig. 4)

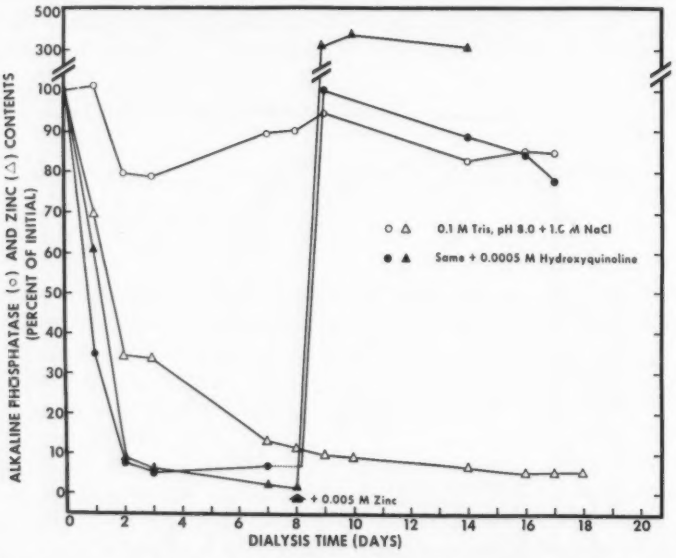
ALKALINE PHOSPHATASE (○) AND ZINC (△) CONTENTS (PERCENT OF INITIAL)
DIALYSIS TIME (DAYS)
○ △ 0.1 M Tris, pH 8.0 + 1.0 M NaCl
● ▲ Same + 0.0005 M Hydroxyquinoline
+ 0.005 M Zinc

FIG. 4. Results of dialysis experiments with oyster alkaline phosphatase. Removal of zinc by dialysis at *p*H 8.0 has little or no effect on enzyme activity (open symbols), whereas dialysis against hydroxyquinoline removes zinc much more effectively and inhibits the enzyme (solid symbols). Addition of zinc completely restores alkaline phosphatase activity.

was typical of preparations stored in the refrigerator for comparable times; it therefore does not represent deactivation by removal of zinc. When oyster extracts are dialyzed against identical buffers containing 0.0005 M 8-hydroxyquinoline, however, the zinc and the alkaline phosphatase decreased concomitantly, and the loss of zinc was over 98% complete after 8 days (Fig. 4). Hydroxyquinoline inhibits metalloenzymes by removing the metal ions from the apoenzyme (Coombs et al., 1962). Addition of excess zinc to the dialysate on the 8th day completely restored alkaline phosphatase activity. Addition of 0.005 M Mn^{++} or Cu^{++} also reversed the inhibition, but the enzyme recovered only to about 20% of its original activity (not shown).

Zinc is removed from oyster extracts also by dialysis against buffers of low *p*H (Fig. 5). The supernatant from an oyster homogenate was diluted fivefold with buffers of the following *p*H: 4.0, 5.0, 6.0 (0.1 M citrate, with 1 M NaCl); and 7.0, 8.0, and 9.0 (0.1 M Tris, with 1 M NaCl). Proteins precipitated immediately upon dilution at *p*H 4 but not at other *p*H's. The dilutions (50 ml) were then dialyzed against 2.4 liters of the same buffer. After 5 days and 7 days

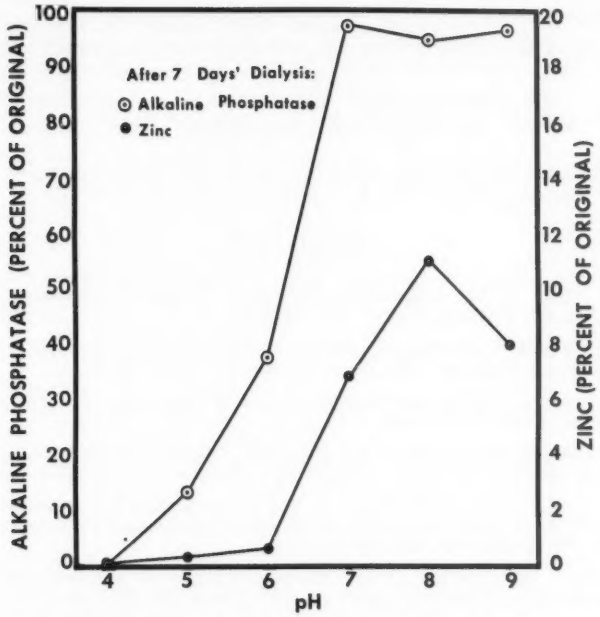

FIG. 5. Removal of zinc and inhibition of alkaline phosphatase by prolonged dialysis of oyster extracts at different *p*H's.

the dialysates were replaced with fresh buffers. The contents of the dialysis sac were sampled periodically and assayed for zinc and alkaline phosphatase. Results of the 7th-day samples are shown in Fig. 5. Although 89–93% of the original zinc was removed at *p*H 7–9, alkaline phosphatase activity was unchanged. This result corresponds also to the data shown in Fig. 4 after about 7 days of dialysis. At *p*H 4–6, loss of zinc was greater and alkaline phosphatase was inhibited markedly; at *p*H 4, it was instantaneous and irreversible. Inhibition by dialysis at *p*H 4.5 could be stopped, but not reversed, by addition of zinc and simultaneous restoration to *p*H 8.0. On the basis of the data shown in Fig. 3–5 and other experimental observations, it appears that zinc is an integral component of oyster alkaline phosphatase.

If alkaline phosphatase is presumed to be representative of zinc enzymes in oysters, it would appear that much of the zinc accumulated by oysters has no metabolic significance. Most of the protein-bound zinc in oysters can be

removed by dialysis with no effect on alkaline phosphatase (Fig. 4 and 5). If, however, more than 95% of the protein-bound zinc is removed, by dialysis either at *p*H 4–6 or in the presence of hydroxyquinoline, alkaline phosphatase activity decreases. Thus, zinc enzymes probably account for only a small portion of the total zinc in oysters. The remaining zinc may be accumulated coincidentally with other ions, like calcium for example. Perhaps the oyster has a poor ionic-discriminator capacity at its environmental interface, and efficient ionic discrimination at the mantle-shell boundary, so that calcium is assimilated and deposited in shell whereas zinc remains loosely bound to tissue proteins.

ACKNOWLEDGMENTS

I appreciate the technical assistance of Jeraldine Brooks, Jo-Ann Lewis, and Twyla Miner. This research was supported by U.S. Atomic Energy Contract AT(49-7)-5.

REFERENCES

BENNETT, R., JR., AND H. I. NAKADA. 1968. Comparative carbohydrate metabolism of marine molluscs. I. The intermediary metabolism of *Mytilus californianus* and *Haliotus rufescens*. Comp. Biochem. Physiol. 24: 787–797.

BEVELANDER, G., AND H. NAKAHARA. 1966. Correlation of lysosomal activity and ingestion by the mantle epithelium. Biol. Bull. (Woods Hole) 131: 76–82.

BODANSKY, M. 1920. Biochemical studies on marine organisms. II. The occurrence of zinc. J. Biol. Chem. 44: 399–407.

COOMBS, T. L., J-P. FELBER, AND B. L. VALLEE. 1962. Metallocarboxypeptidases: mechanism of inhibition by chelating agents, mercaptans, and metal ions. Biochemistry 1: 899–905.

CROSS, F. A., S. W. FOWLER, J. M. DEAN, L. F. SMALL, AND C. L. OSTERBERG. 1968. Distribution of ^{65}Zn in tissues of two marine crustaceans determined by autoradiography. J. Fish. Res. Bd. Canada 25: 2461–2466.

FOLK, J. E., AND E. W. SCHIRMER. 1963. The porcine pancreatic carboxypeptidase A system. I. Three forms of the active enzyme. J. Biol. Chem 238: 3884–3894.

FREEMAN, J. A., AND K. M. WILBUR. 1948. Carbonic anhydrase in molluscs. Biol. Bull. (Woods Hole) 94: 55–59.

GAREN, A., AND C. LEVINTHAL. 1960. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli* I. Purification and characterization of alkaline phosphatase. Biochim. Biophys. Acta 38: 470–483.

GAUMER, H. R. 1966. Blood proteins of the American oyster. (Abstract). Amer. Zool. 6: 332.

HILLER, A., J. PLAZIN, AND D. D. VAN SLYKE. 1948. A study of conditions for Kjeldahl determination of nitrogen in proteins. Description of methods with mercury as catalyst, and titrimetric and gasometric measurements of the ammonia formed. J. Biol. Chem. 176: 1401–1420.

KORRINGA, P. 1952. Recent advances in oyster biology. Quart. Rev. Biol. 27: 266–308.

LAYNE, E. 1957. Spectrophotometric and turbidometric methods for measuring proteins, p. 447–454. *In* S. P. Colowick and N. O. Kaplan [ed] Methods of enzymology, Vol. III. Academic Press, Inc., New York.

MARTIN, A. W. 1961. The carbohydrate metabolism of the mollusca, p. 35–64. I*n* A. W. Martin [ed.] Comparative physiology of carbohydrate metabolism in heterothermic animals. Univ. Wash. Press, Seattle, Wash.

NAKAHARA, H., AND G. BEVELANDER. 1967. Ingestion of particulate matter by the outer surface cells of the mollusc mantle. J. Morphol. 122: 139–146.

PEQUEGNAT, J. E., S. W. FOWLER, AND L. F. SMALL. 1969. Estimates of the zinc requirements of marine organisms. J. Fish. Res. Bd. Canada 26: 145–150.

VALLEE, B. L. 1962. Zinc, p. 443–482. *In* C. L. Comar and F. Bronner [ed.] Mineral metabolism, Vol. II, Pt. B. Academic Press, Inc., New York.

WILBUR, K. M., AND N. G. ANDERSON. 1948. Electrometric and colorimetric determination of carbonic anhydrase. J. Biol. Chem. 176: 147–154.

WOLFE, D. A. 1970. Levels of stable Zn and ^{65}Zn in *Crassostrea virginica* from North Carolina. J. Fish. Res. Bd. Canada 27: 47–57.

WOLFE, D. A., AND T. A. MINER. 1967. Oyster metalloproteins, p. 13–14. *In* Annual report of the Bureau of Commercial Fisheries Radiobiological Laboratory, Beaufort, N.C., for the fiscal year ending June 30, 1966. U.S. Fish. Wildlife Serv. Circ. 270.

WORTHINGTON BIOCHEMICAL CORPORATION. 1968. Carbonic anhydrase (bovine erythrocytes), p. 4.2.1.1. *In* Worthington Manual, April 1968 Revision. Freehold, New Jersey.

Productivity of Macrophytes in Marion Lake, British Columbia[1]

GORDON S. DAVIES[2]

Department of Zoology
University of British Columbia, Vancouver 8, B.C.

DAVIES, G. S. 1970. Productivity of macrophytes in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 27: 71–81.

The in situ productivity of both the phytoplankton and the macrophytes in Marion Lake, B.C., was determined from April 1966 through September 1966, by using ^{14}C techniques. The uptake of $NaH^{14}CO_3$ was measured in selected macrophytes by enclosing them in Plexiglas chambers. These plants were then combusted in oxygen, and the $^{14}CO_2$ was absorbed in toluene-POPOP-ethanolamine. Radioassay was accomplished by liquid scintillation. In addition to the ^{14}C method, an organic weight method was used to measure macrophytic productivity.

The productivity of the macrophytes was always higher than that of the phytoplankton. There was a considerable difference in the estimates of the macrophyte productivity arrived at by the two different methods, and reasons for this are discussed.

The total productivity of the lake is very low when compared with lakes of similar latitudes because of low phytoplankton productivity. It is concluded that in Marion Lake the macrophytes are more important primary producers than the phytoplankton.

The difficulty of comparing data between this and other studies is discussed, and the need for standardization of methods is emphasized.

Received August 21, 1969

INTRODUCTION

IN MOST of the limnological studies dealing with primary productivity, the emphasis has been placed on the productivity of the phytoplankton. Recently Wetzel (1964a, b) and others have noted that in shallow lakes the contribution of the periphyton and higher vascular aquatics may be vastly underrated. The present study attempts to evaluate the role played in primary productivity by the macrophytes of Marion Lake, B.C., and is part of a detailed investigation that will describe the flow of energy through a small freshwater lake.

The primary productivity of this lake for 1965 was recently described by Efford (1967), but since phytoplankton productivity is apt to fluctuate a great deal from year to year, it was again determined in the course of the present study.

The productivity of the macrophytes was determined by making periodic biomass determinations and also by using a ^{14}C method.

---

[1]Canadian Contribution to the International Biological Programme No. CCIBP-46.

[2]Present address: Department of Zoology, University of California, Davis, Calif. 95616, USA.

Printed in Canada (J1572)

METHODS

The Study Area

Marion Lake is located 10 km north of Haney, B.C., in the University Research Forest. The lake lies in a long narrow valley 300 m above sea level, bordered on the west by a steep ridge 300 m above the lake level and on the east by the southern slopes of the Coastal Mountains.

The basin of the lake is 800 m long and about 200 m wide at its maximum. At standard water level the lake has an area of 13.3 ha, one-half of which is less than 2 m deep (Fig. 1). The major inlet stream enters from the north and drains a watershed of 6.5 km² including nearby Eunice Lake. The outlet at the south end feeds into the North Alouette system, which is 1 km away. Both inlet and outlet streams are gravelled, whereas the bottom of the lake consists of a uniform brown ooze.

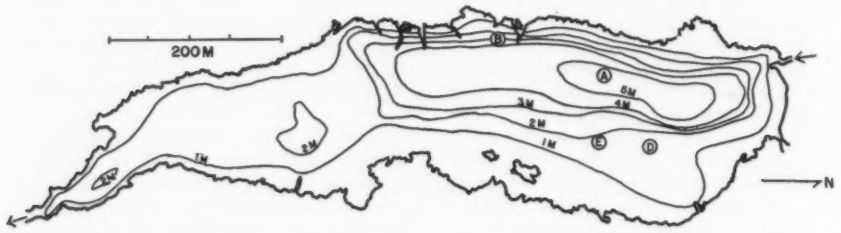

FIG. 1. Morphometric map of Marion Lake, B.C., showing stations A, B, D, and E.

In winter the lake is isothermal, but from spring through fall it is weakly stratified. The winters in the lower mainland of British Columbia are relatively mild and consequently the lake does not regularly have a permanent ice sheet but is sometimes only covered with a thick layer of slush.

A detailed physical description of the lake is given elsewhere (Efford, 1967).

The phytoplankton contains over 200 species (Dickman, MS, 1968) and of these the dominant forms are microflagellates of the Chlorophyceae and Chrysophyceae. Efford (1967) attributes 95% of the phytoplankton productivity to the nannoplankton. He separated the nannoplankton productivity from that of the macroplankton by regularly filtering half a sample normally and half through a #25 net (0.064 mm diam). He further states that spring blooms in the macroplanktonic algae appear not to change productivity significantly.

The periphyton are dominated in the summer by the algae *Mougeotia* spp., which are found in dense mats over the bottom ooze in shallow water and adhering to the stems of *Nuphar polysepala* Engelm. and *Potamogeton natans* L.

There are only four rooted aquatics of numerical significance: *Potamogeton epihydrus* Raf. and *Isoetes occidentalis* Henders. (submerged), and *P. natans* and *N. polysepala* (floating). Dense beds of *Chara globularis* Thuill were found in association with the main spring of the lake and also with several of the smaller ones. *Isoetes occidentalis* is distributed in water over 2 m deep, whereas the other macrophytes are generally found in more shallow areas.

Phytoplankton

To compare the productivity of the phytoplankton with that of the rooted aquatics, the ^{14}C technique introduced by Steeman-Nielsen (1952) and modified by Goldman (1963) was used. Paired samples of water were collected from several depths in a polyethylene bottle and

transferred to clear and opaque glass stoppered bottles. Each sample was inoculated with 4 μc of ^{14}C in the form of $NaH^{14}CO_3$. The samples were then incubated at the depths from which they were obtained for a period of 4–6 hr. This period is considered adequate for significant carbon uptake but brief enough to minimize bottle effects (Vollenweider and Nauwerck, 1961).

Ideally, productivity measurements should have been taken at suitable increments from dawn to dusk each day of the study period, but this was impractical. Instead, a 4-hr midday incubation period was selected to average out the variations in the diurnal curve caused by changing meteorological conditions that could influence the photosynthetic pattern. The assumption was made that productivity is directly proportional to light with full realization that this correlation is not always exact; minimal rates might be obtained, for example, if the measured rates were at maximal intensity. Daily light curves were obtained at Marion Lake with a recording pyrheliometer and the area under these curves was determined by planimetry. The ratio of the area under that portion of the curve representing the incubation period of the total area of the light curve gives a diurnal correction factor that was used to extrapolate daily production values.

After incubation, aliquots of the samples were filtered under vacuum at the lakeside onto HA Millipore filters of a porosity of 0.45 ± 0.02 μ. The activity of the samples was determined at the International Agency for ^{14}C Determination in Denmark.

The method involves difficulties (Thomas, 1961), but it is probably the most sensitive now available for measuring carbon fixation rates under natural conditions and is thought to give values that approximate net productivity (Strickland, 1960).

All observations were made at stations A and E, the assumption being that station A would be typical of that part of the lake deeper than 2 m, and station E of the shallower portion (Fig. 1). The areas represented by A and E were equal, so the productivity of the lake on any given day was taken to be the average of the productivities at A and E.

Rooted Aquatics

There is a vast literature on the investigations of the productivity of higher aquatic plants, much of which has been reviewed by Penfound (1956), Westlake (1963), and Wetzel (1964b). Until recently when Wetzel (1964a) devised a technique for measuring the in situ productivity of aquatic macrophytes with ^{14}C, most of the studies have been concerned with biomass determinations and, less frequently, techniques involving the changes in dissolved oxygen in the water surrounding the plants.

Hartman and Brown (1967) have shown that dissolved oxygen concentrations in the water surrounding vascular plants are not proportional to the production of internal oxygen because of the storage of oxygen in the internal lacunae. Errors involved when using the oxygen technique may be 200% or higher (R. G. Wetzel, personal communication). The above considerations plus the added technical difficulty of obtaining samples while operating at depths of up to 4 m necessitated eliminating the oxygen technique.

Two methods were used to determine the seasonal productivity of the macrophytes; one was based on the calculation of in situ ^{14}C fixation rates, the other on the rate of increase in organic carbon as determined by periodic cropping. In both instances, periodic measurements were made throughout the growing season. Although the organic weight method was used to describe each of the four important macrophytes in the lake, the ^{14}C investigations were carried out only on the two submerged aquatics.

With the aid of scuba, light and opaque Plexiglas chambers were placed over individual plants. The cylinders were similar to those designed by Wetzel (1964a, b) and were calibrated so that the enclosed volume of water was known. Depending on the size of the plants, the volumes used were 1.5 or 2.0 liters. A known amount of ^{14}C (10–20 μc/liter of water) was injected into the chamber as $NaH^{14}CO_3$, by means of a hypodermic syringe. After 4 hr of incubation (10:00 AM until 2:00 PM), the entire plant was removed and epiphytes were stripped from the leaves since they will also fix carbon. The plant was then blotted, weighed, and placed on dry ice.

In the laboratory the plants were dried at 60 C and then ground in a mill until they passed through a #20 mesh (0.25 mm diam) sieve. Ten-mg aliquots of each plant were then combusted

in an oxygen flask designed by Dr C. T. Beer of the Cancer Research Institute of the University of British Columbia (Fig. 2).

When the combustion was complete, the flask was water-cooled to reduce the internal pressure. The capillary tube of the flask was then inserted in a liquid scintillation vial that contained a toluene-POPOP-ethanolamine mixture. When the stopcock of the flask was opened, the scintillation-absorbent mixture was drawn up by the vacuum, into the flask, where it was trapped by closure of the stopcock. The apparatus was agitated intermittently and after 20 min the fluid was returned to the scintillation vial for counting.

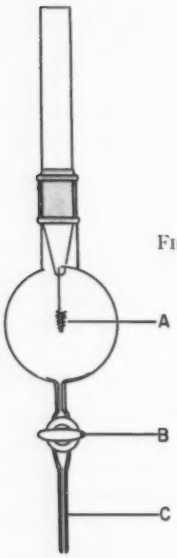

FIG. 2. Oxygen combustion flask showing: A — platinum carriage holding encapsulated sample; B — stopcock; and C — capillary tube.

The efficiency of the above assay (38%) was determined by comparing the counts of replicate samples where one of the plants was placed in an induction furnace, combusted, and the $^{14}CO_2$ evolved flushed into an evacuated 500-ml ionization chamber. This second assay was made with a Dynacon Electrometer that is accurate within 1% by comparison with the American Bureau of Standards samples.

The raw data, which are in counts per minute, are converted to milligrams C in exactly the same way as they are for phytoplankton. To express these values as mg C m^{-2} day^{-1}, it is necessary to know the biomass distribution. All the productivity values given in the paper are expressed as mg C m^{-2} of lake surface.

All observations on *I. occidentalis* were made at station B and those on *P. epihydrus* were made at station D (Fig. 1). On a given sampling day, three light and one opaque chamber measurements were made for each species.

The collection of the biomass data was complicated by the heterogeneous distribution of the plants. Accordingly, with the use of scuba, a thorough map was made of the submergent distribution (Fig. 3). Samples within 1 m^2 quadrats were then collected along transects. Great care was taken to obtain extensive samples for each time period and, further, to obtain the roots and rhizomes where possible as these may represent a considerable portion of the biomass. Scuba greatly facilitated this, although the productivity of *P. natans* may be underestimated because the roots of this plant break very easily.

FIG. 3. Vegetation map of Marion Lake, B.C., 1966. The lake was surveyed with the aid of scuba.

From these samples wet and organic weights were obtained. The organic weight is calculated by subtracting the ash weight from the dry weight. It is assumed to be the best criterion of biomass since it is free from errors due to variable water and ash contents (Westlake, 1963). Carbon analyses were performed on the four species of macrophytes to determine the percentage of organic carbon when related to organic weight. All the values obtained fitted in the range published by Westlake (1963), and it was decided that the value of 47% was representative. This figure, therefore, was used to convert the biomass figures to milligrams C.

The weighing, drying, and ashing methods used agree with those outlined by Westlake (1965a).

RESULTS

The average productivity of the phytoplankton in Marion Lake was 8.04 mg C m^{-2} day^{-1}. The average productivity of the submerged macrophytes was 33.2 mg C m^{-2} day^{-1}, determined by the ^{14}C method. The organic weight method indicated that the combined productivities of the submerged and floating macrophytes was 18.8 mg C m^{-2} day^{-1}, this value being obtained by finding the area under the productivity curve (Fig. 4). All values are corrected for the total lake area.

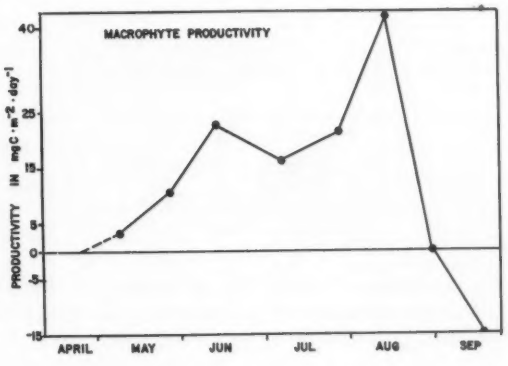

FIG. 4. Productivity of the aquatic macrophytes in Marion Lake, April to September 1966, as determined by organic weight method.

The ^{14}C method of measuring the productivity of the macrophytes yields consistently higher results than does the organic weight method, but no matter which method is considered, the productivity of the macrophytes is always higher than that of the phytoplankton (Table 1). The values shown in the third column of the table represent the ratio of the productivities as measured by the two methods.

TABLE 1. Comparison of productivity (mg C m^{-2} day^{-1}) of macrophytes and phytoplankton in Marion Lake, 1966. Blank values indicate that net respiration equals or exceeds photosynthesis.

	Submerged macrophytes[a]			Total macrophytes[b]	Phytoplankton
Date	^{14}C method	Organic wt method	Ratio	Organic wt method	^{14}C method
May 28	44.2	4.2	11/1	10.8	2.2
June 10	40.7	3.2	12/1	22.9	1.4*[c]
June 30	73.8	2.4	31/1	16.4	2.3*
July 17	31.5	1.7	17/1	21.3	2.2*
Aug. 4	32.9	2.7	12/1	42.1	2.9*
Aug. 18	18.1	2.9	6/1	34.3	2.7*
Sept. 5	2.2	–		–	2.1*
Sept. 15	2.6	–		–	0.8

[a]Submerged macrophytes: *Potamogeton epihydrus* and *Isoetes*.
[b]Total macrophytes: *Potamogeton epihydrus*, *Isoetes*, *Potamogeton* spp., and *Nuphar*.
[c]Values marked with an asterisk (*) are from day closest to sampling date.

Table 2 records, for each of the species of macrophytes, the initial and maximum biomass in grams C m^{-2} of lake surface. A cumulative net productivity is derived for each species at the time when the total organic weight was at a maximum (mid-August). Although the area covered by each species of plant was comparable, it can be seen that the productivity of the floating aquatics is greater than that of the submerged plants by a factor of 10.

TABLE 2. Distribution and cumulative net productivities of the four species of macrophytes in Marion Lake, 1966. Numbers in parentheses represent number of plants involved in determination.

Species	Area % of total	Depth range (*m*)	Biomass (*g C/m²*)		Productivity (*mg C m^{-2} day^{-1}*)
			Initial	Maximum	
Potamogeton natans	7.0	0–2.0	0.5(10)	12.2(10)	7.1
Nuphar polysepala	5.0	0–2.5	4.2(10)	34.2(10)	13.2
Isoetes occidentalis	4.4	1.0–4.0	0.8(10)	4.2(10)	1.3
Potamogeton epihydrus	5.8	0–3.0	0.4(10)	2.2(10)	0.9
Total	22.2		5.9	52.8	22.5

A measure of reliability of the sampling program used in the field, and in the laboratory analyses, is given in Tables 3, 4, and 5. A standard error equal to 10% of the mean is considered tolerable in most ecological studies (Watt, 1968) and it can be seen that this value is never greatly exceeded. Some of the variability in the *P. epihydrus* material may be attributed to the fact that this species grew throughout the time of the study by vegetative means, and it was often a subjective decision as to what constituted an individual plant.

TABLE 3. Variability in numbers of macrophytes between quadrats of 1 m².

Species	No. quadrats counted	Avg no. plants per m²		SE as % of mean	
		May	Sept.	May	Sept.
Isoetes occidentalis	10	9	11	10.8	8.5
Potamogeton epihydrus	10	29	45	8.4	13.6
Potamogeton natans	10	3	16	12.2	5.1

TABLE 4. Variability in fresh weights of individual plants.

Species	No. plants weighed	Avg wt (*mg*)		SE as % of mean	
		Apr.	Aug.	Apr.	Aug.
Potamogeton natans	10	2.4	10.9	2.1	4.7
Nuphar polysepala					
Deep	10	667.4	3644.6	2.3	2.8
Shallow	10	265.0	1812.0	3.4	0.6
Isoetes occidentalis	10	2.6	13.6	3.5	4.5
Potamogeton epihydrus	10	0.4	0.6	17.7	3.8

TABLE 5. Variability in drying and ashing techniques. Each percentage represents a mean value obtained from 10 plants.

Species	Dry wt as % of fresh		SE as % of mean		Ash wt as % of dry		SE as % of mean	
	Apr.	Aug.	Apr.	Aug.	Apr.	Aug.	Apr.	Aug.
Potamogeton natans	15.0	14.1	2.8	1.6	8.2	9.9	2.6	1.7
Nuphar polysepala	10.5	7.7	9.7	7.3	6.3	6.9	10.9	5.9
Isoetes occidentalis	11.2	10.7	8.1	8.1	17.9	22.0	0.8	2.9
Potamogeton epihydrus	13.9	13.0	3.6	4.5	24.0	24.9	11.0	2.9

There was a unimodal temporal pattern in the primary productivity of the phytoplankton in Marion Lake, with the peak of production occurring in early August (Fig. 4 and 5). It is also evident that differences in productivity

between closely occurring days was sometimes considerable. Although the productivity of the macrophytes was based on fewer determinations, a peak was observed in late June followed by a steady decline, until by mid-September there was relatively little productivity (Fig. 5).

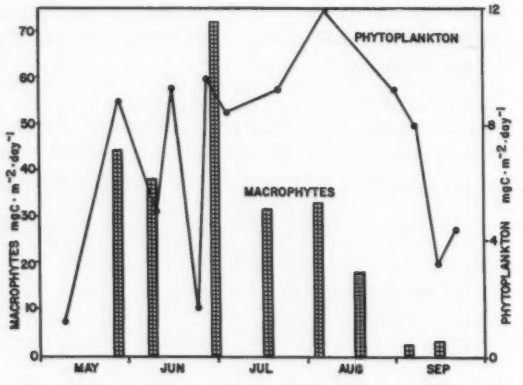

FIG. 5. Comparison of the productivity of the phytoplankton and macrophytes as determined by ^{14}C method in 1966.

The temporal variation in productivity values derived from the organic weight data is somewhat clearer. There is a bimodal curve of productivity with a minor peak occurring in early June followed by a rapid increase in productivity to a major peak in mid-August. The individual curves for three of the species (*P. natans*, *N. polysepala*, and *P. epihydrus* reflect, with minor variation, the overall pattern (Fig. 6). *Isoetes occidentalis* apparently reverses this pattern, exhibiting a major peak in its productivity in late May and a minor peak in mid-August.

DISCUSSION

Marion Lake may be characterized by a very low level of phytoplankton productivity and a relatively high macrophytic productivity. The rates obtained in this study are only estimates, but it is clear that there is agreement between the ^{14}C method and the organic weight method, as to the relative importance of the macrophytes in the lake.

There is, however, considerable difference between the estimates of the productivity of the macrophytes made by the two different methods. The ^{14}C method gives an instantaneous measure of net productivity and there is no correction for respiration in the dark. The organic weight method, since it involves longer periods of time, corrects for rapidly changing environmental conditions that might influence the rate of carbon fixation and also allows for respiration in the dark. This method is considered to give an estimate of net productivity if the community experiences a marked annual fluctuation in its biomass and if it suffers few losses from grazing or death (Westlake, 1963). There is no quantitative evidence from Marion Lake to justify the acceptance

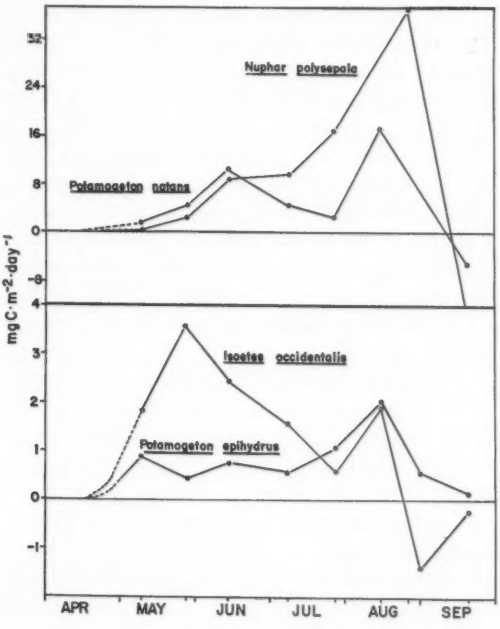

FIG. 6. Comparison of the productivities of the four important macrophytes in Mario Lake, April to September 1966, as determined by the organic weight method.

of the latter two assumptions, but repeated observations indicated that there was little damage done by grazing. According to Westlake (1965a), some aquatic macrophyte communities are scarcely subject to grazing and their production is mostly consumed by bacteria and detritus feeders. This would appear to be the case in Marion Lake, since most of the herbivores found on the plants appeared to be browsing on the attached algae.

It is now clear from a number of phytoplankton studies, where the ^{14}C method has been used, that there is considerable variation in productivity both within days and between days (Rodhe et al., 1958). It is possible, therefore, to explain the lack of a clear temporal pattern in the data, by the infrequent nature of the sampling periods. It is better to sample every day, or where this is not possible, for several consecutive days each month, in order to make a reasonable estimate of productivity. Another source of variability in estimating the productivity of the phytoplankton, arises from the fact that the variability between stations at one time is nearly the same as the variability at one station and one depth over a period of 10 days (Efford, 1967). The two index stations used for the phytoplankton tend to give somewhat higher values than the stations farther south, therefore giving a somewhat exaggerated estimate of the productivity.

The same kind of variability is inherent in the rates determined by measuring the productivity of the macrophytes with ^{14}C. These values are further influenced by the probability that, with few replicates, the experimental plants could be in different physiological states.

The productivity of the phytoplankton in Marion Lake, however, is surprisingly low when compared with that of other temperate zone lakes. Recent unpublished data supports the hypothesis that the rapid flushing of the lake (sometimes within 2 or 3 days) prevents any substantial increase in the phytoplankton population, thereby reducing the rate of carbon fixation (M. D. Dickman, personal communication).

It is very difficult to compare the data from Marion Lake with that obtained from studies on other lakes because of the wide variety of techniques and sampling procedures involved. Westlake (1965b) has recently made an appeal to standardize observational procedures and suggests that one of the main functions of the International Biological Programme should be to recommend methods based on common principles and to obtain internationally comparable observations of the basic biological parameters.

Of all the freshwater productivity studies, there is only one that lends itself to comparison with the Marion Lake study, that of Wetzel (1964b). From his study, Borax Lake is more productive than Marion Lake, but the relative importance of the macrophytic productivity is greater in Marion Lake.

A considerable growth of the periphytic algae, *Mougeotia* spp., was observed in Marion Lake throughout the summer, but their productivities were not monitored owing to the difficulty of placing the experimental chambers over the algae without displacing them or covering them with bottom sediments. It is possible that the periphyton is very important in fixing energy; its importance in supporting a large crop of herbivores has already been observed.

Emergent communities are generally considered to be more productive than submerged ones (Westlake, 1963). Much of the littoral zone of Marion Lake is covered with emergent plants and it is likely that a consideration of their productivity, as well as that of the periphyton, would increase considerably the total estimated productivity. Organic weight criteria would have to be used as no convenient way has yet been devised to measure accurately the in situ carbon fixation for these plants.

ACKNOWLEDGMENTS

I am greatly indebted to Drs I. E. Efford, T. G. Northcote, and D. H. Chitty for their suggestions and encouragement. Dr R. G. Wetzel of Michigan State University offered much good advice and his correspondence was greatly appreciated. Special thanks are due to Mr K. Tsumura who assisted in the field observations and collections, and to Dr M. Dickman and Mr J. Mathias who provided much in the way of help and discussion.

REFERENCES

DICKMAN, M. D. MS, 1968. The relation of freshwater plankton productivity to species composition during induced successions. Ph.D. Thesis. University of British Columbia, Vancouver, B.C.

EFFORD, I. E. 1967. Temporal and spatial differences in phytoplankton productivity in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 24: 2283–2307.

GOLDMAN, C. R. 1963. The measurement of primary productivity and limiting factors in fresh water with carbon-14, p. 103–113. *In* M. S. Doty [ed.] Proc. Conf. Primary Prod. Meas., Mar. Freshwater U.S. AEC Publ. T1D-7633.

HARTMAN, R. T., AND D. L. BROWN. 1967. Changes in internal atmosphere of submersed vascular hydrophytes in relation to photosynthesis. Ecology 48: 252–258.

PENFOUND, W. T. 1956. Primary production of vascular aquatic plants. Limnol. Oceanog. 1: 92–101.

RODHE, W., R. A. VOLLENWEIDER, AND A. NAUWERCK. 1958. The primary production and standing crop of phytoplankton, p. 299–322. *In* A. A. Buzzati-Traverso [ed.] Perspectives in marine biology, Univ. of California Press, Berkeley, Calif.

STEEMAN-NIELSEN, E. 1952. The use of radioactive carbon (C-14) for measuring organic production in the sea. J. Cons. Cons. Perma. Int. Explor. Mer 18: 117–140.

STRICKLAND, J. D. H. 1960. Measuring the production of marine phytoplankton. Fish. Res. Board Can. Bull. 122: 172 p.

THOMAS, W. H. 1961. Physiological factors affecting the interpretation of phytoplankton production measurements, p. 147–162. *In* M. S. Doty [ed.] Proc. Conf. Primary Prod. Meas., Mar. Freshwater U.S. AEC Publ. T1D-7633.

VOLLENWEIDE R. A., AND A. NAUWERCK. 1961. Some observations on the C-14 method for measuring primary production. Verhandl. Int. Ver. Limnol. 14: 134–139.

WATT, K. E. F. 1968. Ecology and resource management. McGraw-Hill Book Co., New York. 450 p.

WESTLAKE, D. F. 1963. Comparisons of plant productivity. Biol. Rev. 38: 385–425.

1965a. Some basic data for investigations of the productivity of aquatic macrophytes. Mem. Inst. Ital. Idrobiol. 18(Suppl.): 229–248.

1965b. Theoretical aspects of the comparability of productivity data. Mem. Inst. Ital. Idrobiol. 18(Suppl.): 313–322.

WETZEL, R. G. 1964a. Primary productivity of aquatic macrophytes. Verhandl. Int. Ver. Limnol. 15: 426–436.

1964b. A comparative study of the primary productivity of higher aquatic plants, periphyton, and phytoplankton in a large, shallow lake. Int. Rev. Ges. Hydrobiol. 49: 1–64.

Principal Acid-Soluble Nucleotides in Adductor Muscle of the Scallop *Placopecten magellanicus* and Their Degradation during Postmortem Storage in Ice

DORIS FRASER HILTZ AND W. J. DYER

Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

HILTZ, D. F., AND W. J. DYER. 1970. Principal acid-soluble nucleotides in adductor muscle of the scallop *Placopecten magellanicus* and their degradation during postmortem storage in ice. J. Fish. Res. Bd. Canada 27: 83–92.

In scallop adductor muscle stored at 0 C, postmortem degradation of adenosine triphosphate (ATP), the predominant nucleotide at death, primarily follows the route ATP → adenosine diphosphate (ADP) → adenosine monophosphate (AMP) → adenosine → inosine → hypoxanthine (Hx). In one lot of scallops, however, inosine monophosphate (IMP) was present as an intermediate along with AMP. Hence, an alternate pathway, similar to that in fish muscle, whereby AMP is degraded to inosine and Hx via IMP rather than adenosine, may exist under certain (undefined) conditions. ATP dephosphorylation was much slower than in fish muscle; however, a rapid turnover of the intermediates in the degradative sequence, indicating relatively high activities of the enzymes catalyzing these reactions, resulted in the formation of appreciable amounts of Hx (3–4 μmole/g) within the edible storage period. In preliminary tests, taste scores correlated well with Hx contents, suggesting that measurement of Hx content may be a useful index of quality in scallop meats.

Column chromatographic analyses showed that adenine nucleotides and their degradation products accounted for about 90% of the total acid-soluble purines and pyrimidines in scallop adductor muscle, with nicotinamide adenine dinucleotide and uridine diphosphate glucose as the principal minor nucleotide constituents. The muscle also contained considerable amounts of homarine (2–3 μmole/g). Glycolysis showed no relation to nucleotide degradation, both processes demonstrating a relatively slow postmortem catabolic rate in scallop muscle.

Received August 6, 1969

INTRODUCTION

NUCLEOTIDE DEGRADATION during chill storage has been investigated extensively in meats (e.g., Bendall, 1960) and in fish (e.g., Jones, 1965; Fraser et al., 1968). In fish muscle, ATP[1] is usually absent at death, having been degraded by muscle ATPase and myokinase to AMP, and thence to IMP by the active fish muscle AMP-deaminating enzyme, AMP-aminohydrolase. During postmortem storage, slower autolysis of the accumulated IMP through Ino to

[1]The following abbreviations are used: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; IMP, inosine 5′-monophosphate; Ino, inosine; Hx, hypoxanthine; NAD, nicotinamide adenine dinucleotide; UDPG, uridine diphosphate glucose; UDPAG, uridine diphosphate N-acetyl-glucosamine.

Printed in Canada (J1555)

Hx is the reaction of interest. Hx accumulates from the moment of death, and provides a useful index of storage time in ice, and of eating quality, for many fish species (see reviews by Burt et al., 1969; Hiltz et al., 1969).

By comparison, marine invertebrate species have been little studied. Saito et al. (1958), and subsequently Arai (1966a), indicated that the nucleotide degradative pathway in marine molluscs may differ appreciably from that in fish and higher vertebrates, concluding that the major route for conversion of AMP to Ino was via adenosine rather than IMP. In crustacean muscle, however, a relatively weak AMP-aminohydrolase activity, as demonstrated in the lobster (Dingle et al., 1968), results in the formation of some IMP.

Our purpose was to identify the principal nucleotides in the (edible) adductor muscle of scallop (*Placopecten magellanicus*) immediately post-mortem, and to follow their subsequent changes during storage in ice. We also wished to assess the possible use of one or more of the degradation products as an index of quality for this commercially important east coast species.

MATERIALS AND METHODS

Processing, Sampling, and Extraction

Live scallops from the extensive fishery on Georges Bank were either processed within a day of landing or were held at the laboratory, without feeding, in filtered, re-circulated sea water at 6–10 C until required. As the scallops were shucked, the adductor muscles were rinsed with ice-cold 3% NaCl solution, drained briefly, packaged immediately in polyethylene bags, and buried in ice. No more than 36 meats were placed in a single layer in each bag.

For sampling, a slice 5–10 mm wide was cut across the adductor muscle, parallel to the muscle fibres, from each of 4–6 scallop meats and pooled for a composite sample (15–30 g) for extraction. Similar composite samples from the same 4–6 scallops were used for *p*H determination. Care was taken to exclude the small, yellowish, "catch" muscle attached to the adductor. Initial samples were extracted within 1 hr of shucking.

Nucleotides and associated metabolites were extracted by homogenizing the samples at 0–3 C with 2 volumes of ice-cold 6% perchloric acid for 3 min, followed by centrifugation and washing of the residue with 6% perchloric acid. The combined supernatant solutions were filtered through acid-washed glass wool, neutralized to *p*H 6.5–7.0 with 30% KOH, and held 30 min at 0 C before removal of the potassium perchlorate. The neutralized extracts were stored at 4 C for use within 24 hr, or were frozen at −40 C to await analysis.

Column Chromatography

Neutralized perchloric acid extract was applied directly to a jacketed (0–2 C) column (18 × 1.8 cm) of Dowex 1 × 8 (formate), 200–400 mesh, and the column washed with water until the effluent was free of 260 nm-absorbing material. Chromatography was carried out with a formic acid–ammonium formate system of stepwise gradient elution, based on the procedure of Hurlbert et al. (1954); initial elution with a range of 0–0.5 N formic acid followed by 2 N formic acid improved the resolution of the earlier peaks. The column effluent was monitored continuously during loading, washing, and elution by an ultraviolet absorption meter equipped with a dual interference filter of output band approximately 20 nm wide, sharply peaked at 265 nm (Gilson Medical Electronics, Middleton, Wis.).

Fractions common to individual peaks were pooled, and identification and quantitative evaluation of the base moiety of the nucleotides made by the differential adsorption-spectrophotometric assay procedure of Adler et al. (1967), using established molar extinction coefficients as well as 250/260 nm absorbing ratios. For further identification, the pooled fractions were freeze-dried for thin-layer chromatographic analysis (see below) by comparison with known markers.

Thin-Layer Chromatography (TLC)

A simple TLC technique (Fraser et al., 1967) was used to separate the major nucleotides, nucleosides, and bases, and to monitor postmortem changes in these metabolites. Neutralized $HClO_4$ extract, containing 0.02–0.03 μmoles of purine compounds, was applied directly to the poly(ethyleneimine)-cellulose-coated plastic sheets.

Analytical Methods

Contents of ATP, ADP, AMP, and IMP in the neutralized muscle extracts were determined after TLC by quantitative elution of the spots with 0.6 ml of 2 M NaCl for 1 hr at room temperature, followed, after sedimentation of particles, by spectrophotometric examination. $E^{pH\ 7,\ 1\ cm}_{260\ nm} = 13780$ was used for adenine nucleotides and $E^{pH\ 7,\ 1\ cm}_{251\ nm} = 11500$ for IMP (J. R. Dingle, personal communication). In later experiments, adenine nucleotide concentrations were also determined enzymically using the reagent kits supplied by Boehringer-Mannheim Corp., New York. These values and those obtained by elution from thin-layer chromatograms agreed within 10–12%.

Hypoxanthine was estimated enzymically with xanthine oxidase (Jones et al., 1964). This assay also measures xanthine; however, no xanthine was detected in scallop muscle immediately post-mortem or during iced storage when estimated by differential spectrophotometry (Kalckar, 1947).

A new procedure for Ino estimation was developed, based on the difference between the Hx value obtained after hydrolysis of the ribosidic linkage with acid (0.5 N H_2SO_4 at 100 C for 1 hr and neutralization to *p*H 7.0–7.5) (Kalckar, 1947) and that measured before hydrolysis (pre-formed Hx due to endogenous riboside hydrolyase activity). Since we also found IMP to be approximately 75% hydrolyzed under these conditions, the extracts must be shaken with Dowex 1 (Cl^-) to remove nucleotides (Jones and Murray, 1964) before Ino hydrolysis is carried out. Recoveries of Ino and of mixtures of Ino and Hx added to extracts before Dowex treatment and before hydrolysis ranged from 95 to 102%.

Homogenates of muscle samples with 5–8 volumes of 0.005 M sodium iodoacetate (Marsh, 1952) were used for *p*H determination.

Glycogen is reported as the sum of the glycogen in the unneutralized $HClO_4$ extract and in a KOH digest of the residue (Nowlan et al., 1968).

RESULTS

Column Chromatographic Separation of the Major Acid-Soluble Nucleotides (Fig. 1)

Peak 1*a*, representing nonnucleotide material eluted during the loading and water-washing, was predominantly homarine, a quaternary ammonium base widely distributed among invertebrates (Gasteiger et al., 1960). To separate contaminants of the homarine fraction (indicated by nontypical absorption ratios), the pooled fractions of the peak were dried, reconstituted in water, and re-chromatographed on AG50W × 8 (H^+) resin (Calbiochem, Los Angeles), using a linear gradient of 2 N HCl (500 ml) into water (500 ml). This procedure effectively separated homarine, identified by spectral properties identical with those of the pure compound, from amino acid-type material not held by the resin and from other, as yet unidentified, interferences contained in a second peak eluted after homarine.

Peak 1*b*, also representing material not held by Dowex 1 (formate), was not detected in initial extracts, but appeared in appreciable quantity after 6 days in ice. The absorption spectrum indicated inosine-type compound(s), identified by TLC as Ino and Hx, present in about equal amounts.

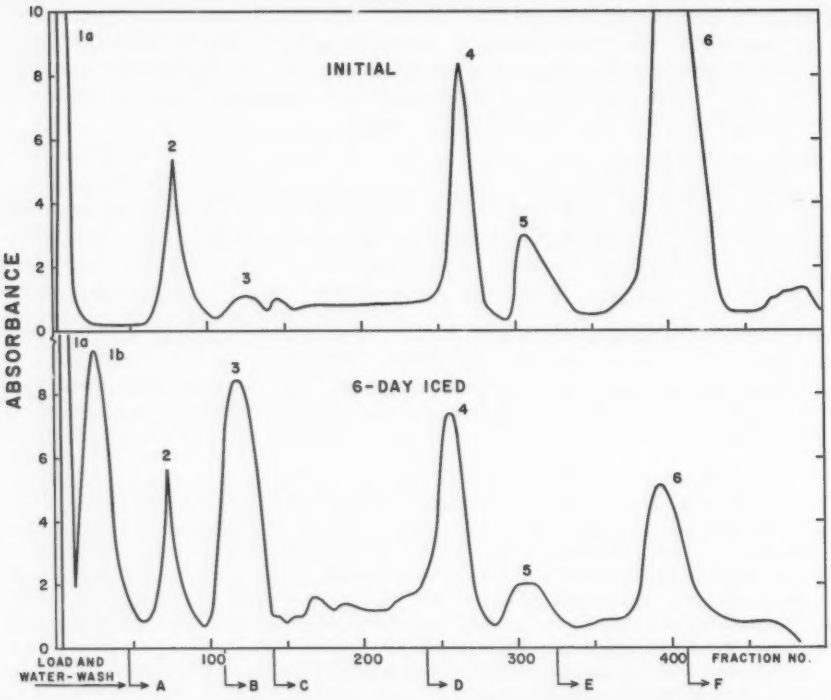

FIG. 1. Column chromatographic separation of the acid-soluble nucleotides of scallop adductor muscle immediately post-mortem and after 6 days in ice. Both samplings were from the same lot of scallops. The peaks were identified as: 1*a*, homarine; 1*b*, Ino + Hx; 2, NAD; 3, AMP; 4, ADP; 5, UDPG + trace of UDPAG; 6, ATP. Extracts, equivalent to 15.4 and 11.5 g muscle (initial and 6-day, respectively) were chromatographed on Dowex 1 by nonlinear gradient elution with formic acid–ammonium formate buffers; fraction size was 10 ml. Absorbance (arbitrary units) was monitored by an ultraviolet absorption meter, output band 20 nm wide, peaked sharply at 265 nm. The eluting solvents were: A, 0.5 N formic acid; B, 2 N formic acid; C, 4 N formic acid; D, 4 N formic acid + 0.2 M ammonium formate; E, 4 N formic acid + 0.4 M ammonium formate; F, 4 N formic acid + 0.8 M ammonium formate. See text for details.

Peaks 2, 3, and 4 were identified by spectral and thin-layer chromatographic properties as NAD, AMP, and ADP respectively. The identification of peak 2 as NAD was confirmed by the cyanide reaction (Ciotti and Kaplan, 1957).

Peak 5 contained UDPG contaminated with a small amount of UDPAG. These were identified by spectral characteristics, and by the release of UMP on acid hydrolysis in 1 N HCl and of UDP on mild acid hydrolysis (0.01 N HCl). Paper chromatograms in isopropanol:H_2O (Smith, 1960), sprayed with aniline hydrogen phthalate, showed the latter hydrolyzate to contain glucose, with traces of another sugar that responded weakly to the *p*-dimethylaminobenzaldehyde reaction for N-acetyl-glucosamine (Partridge, 1948).

Peak 6 was identified as ATP, with a trace of ADP revealed by TLC, probably as a result of slight decomposition of ATP during eluent removal.

ATP accounted for about 85% of the total nucleotide content of freshly killed scallop muscle (Table 1). Within 6 days storage in ice, 90% of this high-energy metabolite had been degraded, with concomitant increases in AMP, Ino, and Hx. The loss in total nucleotide in 6 days (3.26 μmoles/g) was accounted for entirely by the accumulation of Ino and Hx (3.38 μmoles/g). About the same levels of ADP, NAD, and the nucleotide sugars were present after 6 days in ice as in the initial samples. The difference in homarine content may reflect variation among individual scallop meats.

TABLE 1. Acid-soluble nucleotides and associated compounds, separated by column chromatography (Fig. 1), in scallop muscle immediately post-mortem and after 6 days in ice.

Component	Content in muscle (μmoles/g)	
	Initial	6-day iced
ATP	5.30	0.52
ADP	0.65	0.76
AMP	Trace	1.40
NAD	0.08	0.12
UDPG + UDPAG	0.20	0.17
Total nucleotide	6.23	2.97
Ino + Hx[a]	None	3.38
Homarine[b]	1.59	2.56

[a]Calculation based on a molar extinction coefficient (1 cm, pH 7) of 9700 at 254.5 nm, the isobestic point for mixtures of Ino and Hx.

[b]Calculated from $E^{1cm}_{272\ nm}$ = 6325 (Gasteiger et al., 1960).

DEGRADATION OF ADENINE NUCLEOTIDES DURING POSTMORTEM STORAGE IN ICE

To extend the column chromatographic findings of changes in 6 days in ice, the pathway of postmortem ATP degradation in scallop muscle at 0 C was monitored at frequent intervals by TLC (Fig. 2). The most dramatic finding was the almost complete dephosphorylation of ATP, with appreciable accumulation of AMP, within the first 2 days. After the initial drastic decrease, traces of ATP remained throughout the storage period, as did traces of ADP; this stable adenine nucleotide fraction probably represents nucleotide bound to myofibrillar protein (Perry, 1952). Quantitative evaluation of the TLC components (Fig. 3) revealed that AMP accumulated in 2 days to a maximum equivalent to about 50% of the ATP present initially; Ino accumulation was relatively small, only 1–2 μmoles/g; and Hx formation, quite rapid in the early stages, reached levels of 2–3 μmoles/g in a week and thereafter slowed, with maximum levels not attained even at 18 days. IMP (or adenosine) was

not detected by TLC (the limit of detection under the TLC conditions used was about 0.2 μmole/g), nor was it a constituent of the muscle analyzed by column chromatography (Fig. 1).

Accompanying the ATP dephosphorylation, muscle *p*H fell sharply (insert, Fig. 3). Surprisingly, glycogen levels did not change significantly over the storage period; the average value ± SE was 2.88% ± 0.14 for eight determinations, with a range of 2.28–3.65%.

Although several other lots of scallops exhibited the same sequence of nucleotide degradative changes depicted in Fig. 2 and 3, considerable differences in degradative rate were observed (Fig. 4). Differences among the various lots of scallops were also noted in initial glycogen content, in the extent of glycogen breakdown during storage, and in the ultimate *p*H reached. However, there did not appear to be an obvious relation between any one of these factors and the rate of nucleotide degradation.

Unexpectedly, in one of the several lots of scallops examined (experiment 2), the TLC analysis revealed a strikingly different pattern of degradative changes: IMP was present, in low yet consistent amounts (up to 1 μmole/g), throughout the 17-day storage period (Fig. 5). In this experiment too, unlike others discussed above, the content of AMP did not at any time exceed 1 μmole/g and only traces of Ino accumulated.

DISCUSSION

ATP is clearly the predominant nucleotide in scallop adductor muscle at death, and its dephosphorylation through ADP to AMP constitutes the first step in the postmortem nucleotide degradative pathway. The rate of dephosphorylation was relatively slow (from 2 to 9 days for completion) when compared with fish muscle, in which high initial ATP levels can be maintained for as long as 24 hr post-mortem only under conditions of complete muscle relaxation at death, e.g., through anaesthesia (Fraser et al., 1967). The accumulation of the intermediate AMP is also in sharp contrast to fish and mammalian muscle, where IMP accumulates in stoichiometric relation to the ATP lost (Bendall, 1960; Fraser et al., 1967). In the scallop, ATP dephosphorylation to AMP was always accompanied by a fall in muscle *p*H. Apparently, this early *p*H decrease was not due to glycolysis, as in fish muscle, since glycogen was not always degraded in the scallop. Instead, we postulate that it may result from the release of phosphoric acid when ATP is hydrolyzed to AMP. In fish muscle, this would be partially counteracted by the neutralizing effect of the ammonia produced in the simultaneous deamination of AMP to IMP.

Our results do not indicate whether the subsequent steps in the degradative sequence — decay of AMP through Ino to Hx — proceed in scallop muscle primarily via IMP or adenosine. The latter pathway is favored, since strong adenosine aminohydrolase activity and the apparent absence of AMP aminohydrolase have recently been demonstrated in scallop muscle extracts (J. R. Dingle and J. A. Hines, unpublished data). Muscle of the Japanese scallop,

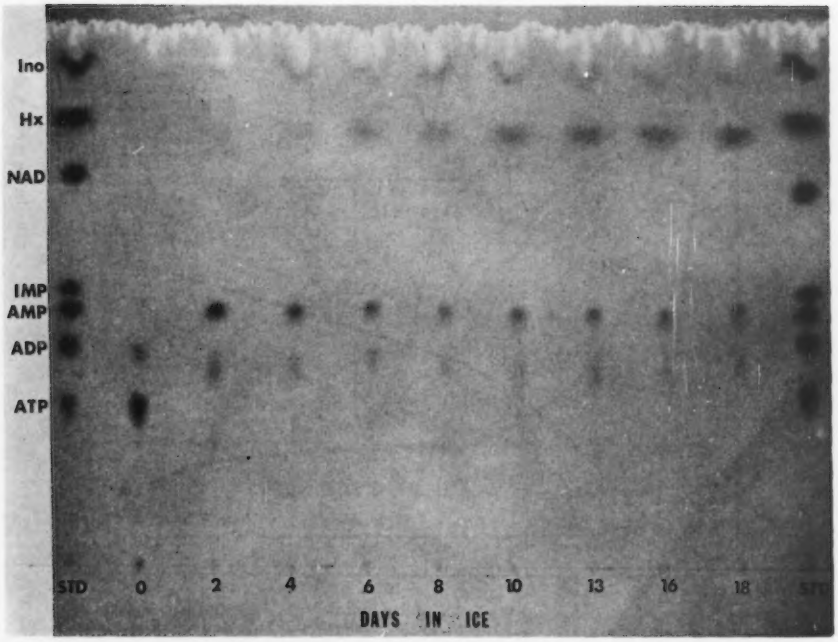

FIG. 2. Thin-layer chromatogram of the progression of nucleotide degradative changes in scallop muscle at 0 C (experiment 6, Fig. 4, typical of several lots examined). PEI — cellulose layer; photographed under ultraviolet light.

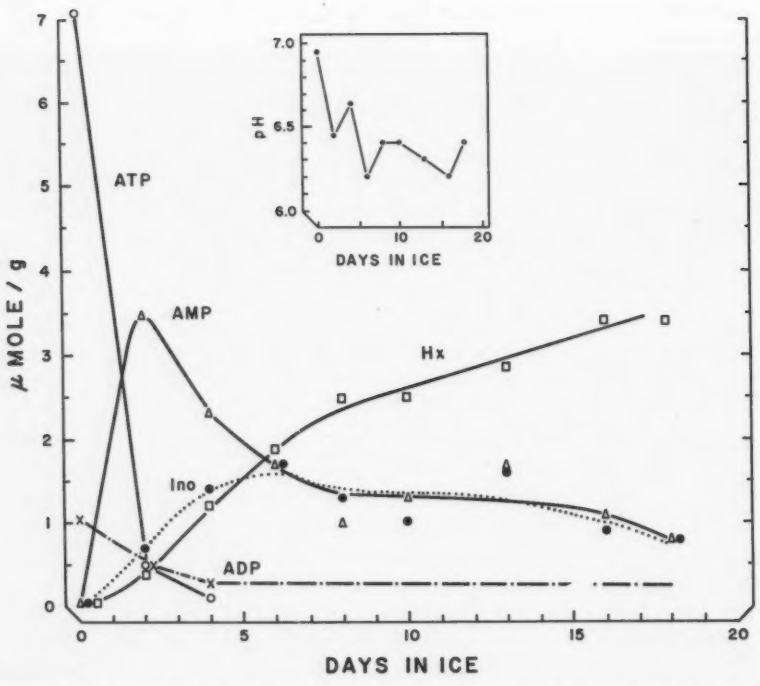

FIG. 3. Degradation of adenine nucleotides to Ino and Hx in scallop muscle stored in ice. Experimental material the same as for Fig. 2. Each point represents a composite sampling of six different scallop meats. ATP, ADP, and AMP determined by quantitative elution from thin-layer plates (Fig. 2); Ino and Hx determined enzymically in neutralized $HClO_4$ extracts with xanthine oxidase. Insert: The accompanying changes in muscle *p*H.

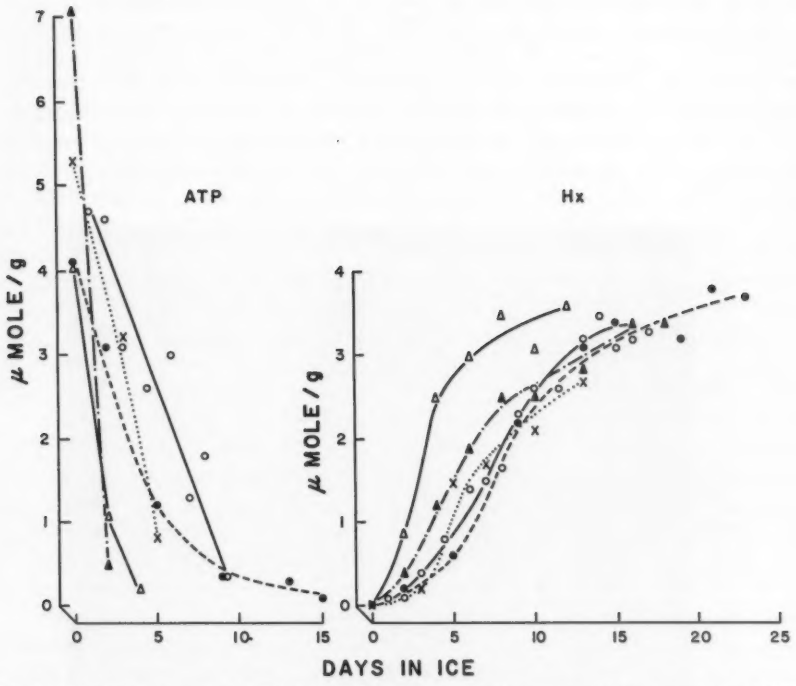

FIG. 4. Variation in rate of ATP dephosphorylation and Hx accumulation among five different lots of scallop meats during storage in ice.

Expt. no.	Month caught	Live-holding time[a]	Initial glycogen content (%)	Glycogen metabolized[b]	Ultimate *p*H reached
1 (●—●)	May	1 month	1.6	Yes	6.7
2 (○—○)	Sept.	3 months	0.2	Yes	6.85
3 (△—△)	Apr.	13 days	0.9	Yes	6.7
5 (X . . . X)	June	4 days	3.0	No	6.3–6.4
6 (▲- -▲)	June	1–2 days	3.0	No	6.2–6.4

[a]The time held alive, without feeding, in laboratory aquaria at 6–10 C before shucking and storage in ice.

[b]A progressive decrease in glycogen content to about 10% or less of the initial level over the storage period held.

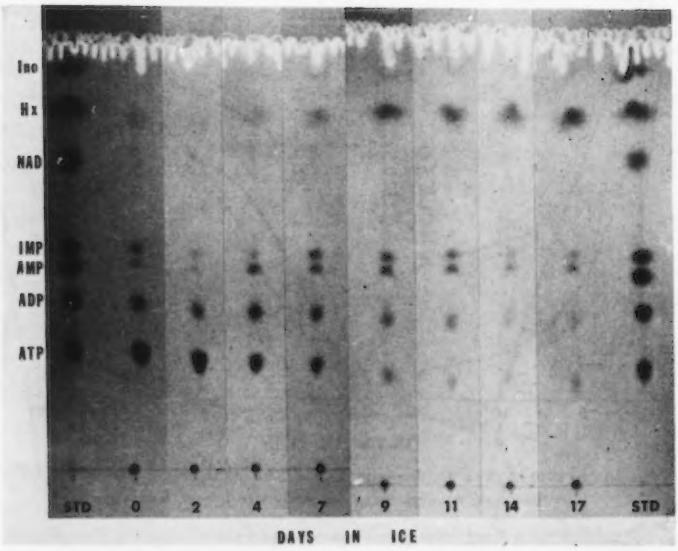

FIG. 5. Thin-layer chromatogram of the progression of nucleotide degradative changes in one particular lot (experiment 2, Fig. 4) of iced scallops, showing the presence of IMP.

Pecten yessoensis, with similar aminohydrolase activities, is also reported to degrade AMP by this route (Arai, 1966b). However, the occurrence of IMP in one lot of scallops examined is evidence for an alternate pathway, probably involving deamination of AMP to IMP with subsequent dephosphorylation to Ino; it indicates that *P. magellanicus* muscle does not lack AMP-aminohydrolase, but that particular conditions, as yet undefined, are apparently required for stimulation of its activity, though the particular lot involved may represent an aberrant population. These scallops (experiment 2, Fig. 4) were caught in September, during the spawning season (Bourne, 1964), had a low glycogen content, and were undoubtedly starved when killed; ATP dephosphorylation was very slow (Fig. 5), and the *p*H remained high, ≥ 6.85. Possibly one or more of these factors, peculiar to this lot of scallops, may have affected the rate and extent of enzyme activity. IMP, along with AMP, has been found in another lamellibranch, the Pacific oyster, *Crassostrea gigas* (Wylie and Smith, 1964), but not in several molluscan species investigated by Arai (1966a). In crustaceans, the two nucleotide monophosphates are commonly present in the muscle post-mortem (Tarr and Comer, 1965; Arai, 1966a; Porter, 1968).

The formation of only small amounts of Ino, 1–2 μmoles/g, indicated that Ino ribohydrolase in scallop muscle was relatively more active than the rate-limiting 5′-ribonucleotide phosphohydrolase. In contrast, Ino ribohydrolase activity in *Pecten yessoensis* is apparently much weaker, since up to 5 μmoles/g of Ino were formed post-mortem prior to Hx accumulation (Arai, 1960); this emphasizes the wide variation in enzyme properties that may exist even among similar species.

As a result of the fairly rapid turnover of intermediates in the degradative sequence, Hx was formed in appreciable quantity early in the storage period, within the confines of edibility (Fig. 3 and 4). To test the value of Hx content as a quality index in scallops, taste scores, assigned by an experienced taste panel (Dyer et al., 1964), were plotted against Hx contents; the resulting regression line (Fig. 6) showed a highly significant ($P < 0.001$) linear relation. The limit of acceptability (taste score of 40) corresponds to a Hx content of 3.7 μmoles/g, about the maximum usually reached (Fig. 4). Hence, samples with a Hx content of 3 μmoles/g or greater would indicate a quality, at best, at or near the unacceptability level. Further study is necessary, especially under a variety of practical conditions, to appraise fully the potentiality of Hx content as a quality index in scallop meats. It should be noted that both AMP and IMP may contribute a meaty flavor (Hashimoto, 1965), and their disappearance may lead to loss of flavor.

Column chromatographic analysis revealed NAD, and UDP sugars (mostly UDPG), as minor acid-soluble nucleotide constituents of scallop muscle. NAD levels are similar to those reported for rested muscle of cod (0.1 μmole/g) (Jones and Murray, 1960), but UDPG contents are considerably higher, only traces being found in cod. UDPG is an intermediate reactant in glycogen synthesis (Leloir et al., 1959); its presence in more than trace amounts in scallop muscle suggests that the synthesis of glycogen in this species may involve

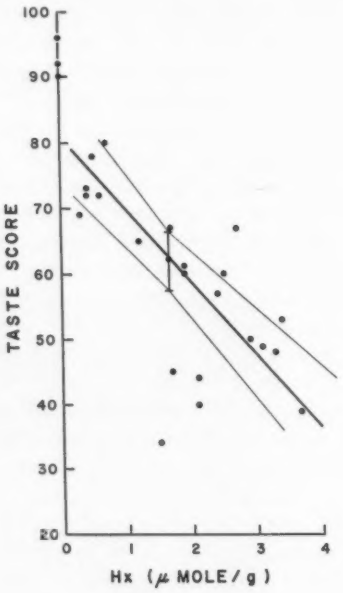

FIG. 6. Regression line of taste scores on Hx contents in several lots of scallop meats stored in ice. 95% confidence intervals of the mean are shown; Y = 81–11X (n = 25). The meats were cooked by steaming and evaluated by an experienced taste panel according to established procedures. The numerical scoring system used ranges from 100 (perfect) to 0 (badly spoiled quality), with the limit of acceptability about 40. Each point represents the average of 24 tastings.

UDPG-glycogen glucosyltransferase rather than phosphorylase. Invertebrate muscle phosphorylase, e.g., in lobster tail muscle (Cowgill, 1959), is apparently much less active than the mammalian (rabbit) enzyme. In scallop muscle, a low phosphorylase activity is suggested by the relative stability of glycogen post-mortem.

The amounts of homarine found (1.6–2.6 μmoles/g) are within the range reported for *Pecten* sp. by Gasteiger et al. (1960). These quantities may be sufficient to interfere with the simpler estimations of purine compounds in the $HClO_4$ extracts by direct spectrophotometric measurement, sometimes used as an index of quality in fish muscle (Jones and Murray, 1964; Fraser et al., 1968).

The preliminary data on glycogen content in scallop muscle revealed several interesting points worthy of further investigation. Initial glycogen levels varied more than 10-fold among the several lots of scallops examined (Fig. 4), probably as a result of sexual maturity, nutritional condition, or season. Curiously, glycogen was degraded in some lots of scallops during postmortem storage, and not in others, and our limited data suggest that *p*H may be of principal importance in this. Glycolysis did proceed when muscle *p*H's of 6.7 and above were maintained post-mortem, but no significant glycogen breakdown occurred in two experiments where low ultimate *p*H levels (6.2–6.4) were reached within 2–3 days. In the latter, inhibition at low *p*H (Danforth, 1965) of the activities of phosphorylase or phosphofructokinase, or both, the two key enzymes implicated in the regulation of glycolysis post-mortem

(Newbold and Scopes, 1967), may be involved. In those scallops where glycogen degradation did occur post-mortem, the rate of decrease was much slower (up to 21 days for 90% degradation) than that found even in relaxed cod muscle at 0 C (complete in 4 days) (Fraser et al., 1967). This, along with the slower nucleotide catabolism, demonstrates a relatively slow overall postmortem catabolic rate in scallop muscle.

ACKNOWLEDGMENTS

We thank Miss T. E. Lightle for skilled technical assistance in certain phases of this work. The scallops were obtained through the excellent cooperation of Mr F. C. Read, Lunenburg Sea Products, Lunenburg, N.S.

REFERENCES

ADLER, N. A., G. J. LITT, AND R. G. JOHL. 1967. Differential adsorption-spectrophotometric assay method for nucleotides. Anal. Chem. 39: 249–251.

ARAI, K. 1960. I. Acid-soluble nucleotides in muscle of marine invertebrates. Effects of storing temperature upon the content of muscular nucleotides of some seashells. Bull. Fac. Fish. Hokkaido Univ. 11: 67–72.

1966a. Nucleotides in marine invertebrate muscle. Bull. Jap. Soc. Sci. Fish. 32: 174–179.

1966b. V. Acid-soluble nucleotides in muscle of marine invertebrates. Degradation of adenylic acid in the muscles of scallop and abalone. Bull. Fac. Fish. Hokkaido Univ. 17: 91–98.

BENDALL, J. R. 1960. Postmortem changes in muscle, p. 227–274. *In* G. H. Bourne [ed.] The structure and function of muscle. Vol. 3. Academic Press, Inc., New York.

BOURNE, N. 1964. Scallops and the offshore fishery of the Maritimes. Fish. Res. Bd. Canada Bull. 145: 60 p.

BURT, J. R., G. D. STROUD, AND N. R. JONES. 1969. Estimation of hypoxanthine concentrations in fish muscle by a rapid, visual modification of the enzymatic assay procedure, p. 367–370. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Ltd., London.

CIOTTI, M. M., AND N. O. KAPLAN. 1957. Procedures for determination of pyridine nucleotides, p. 890–899. *In* S. P. Colowick AND N. O. Kaplan [ed.] Methods in enzymology. Vol. 3. Academic Press, Inc. New York.

COWGILL, R. W. 1959. Lobster muscle phosphorylase: purification and properties. J. Biol. Chem. 234: 3146–3153.

DANFORTH, W. H. 1965. Activation of glycolytic pathway in muscle, p. 287–297. *In* B. Chance, R. W. Estabrook, AND J. R. Williamson [ed.] Control of energy metabolism. Academic Press, Inc., New York.

DINGLE, J. R., J. A. HINES, AND D. I. FRASER. 1968. Postmortem degradation of adenine nucleotides in muscle of the lobster, *Homarus americanus*. J. Food Sci. 33: 100–103.

DYER, W. J., D. I. FRASER, R. G. MACINTOSH, AND M. MYER. 1964. Cooking method and palatability of frozen cod fillets of various qualities. J. Fish. Res. Bd. Canada 21: 577–589.

FRASER, D. I., J. R. DINGLE, J. A. HINES, S. C. NOWLAN, AND W. J. DYER. 1967. Nucleotide degradation, monitored by thin-layer chromatography, and associated postmortem changes in relaxed cod muscle. J. Fish. Res. Bd. Canada 24: 1837–1841.

FRASER, D. I., D. P. PITTS, AND W. J. DYER. 1968. Nucleotide degradation and organoleptic quality in fresh and thawed mackerel muscle held at and above ice temperature. J. Fish. Res. Bd. Canada 25: 239–253.

GASTEIGER, E. L., P. C. HAAKE, AND J. A. GERGEN. 1960. An investigation of the distribution and function of homarine (N-methyl picolinic acid). Ann. N.Y. Acad. Sci. 90: 622–636.

HASHIMOTO, Y. 1965. Taste-producing substances in marine products, p. 57–61. *In* R. Kreuzer [ed.] The technology of fish utilization. Fishing News (Books) Ltd., London.

HILTZ, D. F., W. J. DYER, S. NOWLAN, AND J. R. DINGLE. 1969. Processing, handling and environmental factors, species differences and freezing effects with regard to applicability of various quality indices based on nucleotide degradation. FAO Tech. Conf. on Fish Inspection and Quality Control, Halifax, N.S., July, 1969.

HURLBERT, R. B., H. SCHMITZ, A. F. BRUMM, AND V. R. POTTER. 1954. Nucleotide metabolism II. Chromatographic separation of acid-soluble nucleotides. J. Biol. Chem. 209: 23–39.

JONES, N. R. 1965. Interconversions of flavorous nucleotide catabolites in chilled and frozen fish. Proc. Int. Congr. Refrig. XIth, Munich, 1963. Vol. II. Pergamon Press, London. p. 917–922.

JONES, N. R., AND J. MURRAY. 1960. The acid-soluble nucleotides of codling (*Gadus callarias*) muscle. Biochem. J. 77: 567–575.

1964. Rapid measures of nucleotide dephosphorylation in iced fish muscle. Their value as indices of freshness and of inosine 5′-monophosphate concentration. J. Sci. Food Agr. 15: 684–690.

JONES, N. R., J. MURRAY, E. I. LIVINGSTON, AND C. K. MURRAY. 1964. Rapid estimations of hypoxanthine concentrations as indices of the freshness of chill-stored fish. J. Sci. Food Agr. 15: 763–774.

KALCKAR, H. M. 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. I. Determination of hydroxypurine compounds. J. Biol. Chem. 167: 429–443.

LELOIR, L. F., J. M. OLAVARRIA, S. H. GOLDEMBERG, AND H. CARMINATTI. 1959. Biosynthesis of glycogen from uridine diphosphate glucose. Arch. Biochem. Biophys. 81: 508–520.

MARSH, B. B. 1952. Observations on rigor mortis in whale muscle. Biochim. Biophys. Acta 9: 127–132.

NEWBOLD, R. P., AND R. K. SCOPES. 1967. Postmortem glycolysis in ox skeletal muscle. Effect of temperature on the concentrations of glycolytic intermediates and cofactors. Biochem. J. 105: 127–136.

NOWLAN, S. S., W. J. DYER, AND D. I. FRASER. 1968. Problems involved in the quantitative extraction of glycogen and high-energy phosphorus compounds in frozen cod muscle. J. Fish. Res. Bd. Canada 25: 1525–1538.

PARTRIDGE, S. M. 1948. Filter paper partition chromatography of sugars. 1. General description and application to the qualitative analysis of sugars in apple juice, egg white and foetal blood of sheep. Biochem. J. 42: 238–250.

PERRY, S. V. 1952. The bound nucleotide of the isolated myofibril. Biochem. J. 51: 495–499.

PORTER, R. W. 1968. The acid-soluble nucleotides in king crab muscle. J. Food Sci. 33: 311–314.

SAITO, T., K. ARAI, AND T. TANAKA. 1958. Changes in adenine nucleotides of squid muscle. Nature 181: 1127–1128.

SMITH, I. 1960. Sugars, p. 246–260. *In* I. Smith [ed.] Chromatographic and electrophoretic techniques. Vol. 1. Interscience Publishers, Inc., New York.

TARR, H. L. A., AND A. G. COMER. 1965. Nucleotides and related compounds, sugars, and homarine in shrimp. J. Fish. Res. Bd. Canada 22: 307–311.

WYLIE, V., AND M. SMITH. 1964. Nucleotides of the Pacific oyster *Crassostrea gigas* (Thunberg). Can. J. Biochem. 42: 1347–1351.

Effects of the Insecticide Sevin on Various Stages of the Dungeness Crab, *Cancer magister*[1]

DAVID V. BUCHANAN, RAYMOND E. MILLEMANN, AND NELSON E. STEWART

Department of Fisheries and Wildlife
Marine Science Center, Oregon State University
Newport, Oreg. 97365, USA

BUCHANAN, D. V., R. E. MILLEMANN, AND N. E. STEWART. 1970. Effects of the insecticide Sevin on various stages of the Dungeness crab, *Cancer magister*. J. Fish. Res. Bd. Canada 27: 93–104.

Of the life-history stages of the Dungeness crab, *Cancer magister*, the early larvae are more sensitive to Sevin than are juveniles and adults. A concentration of 1.0 mg/liter did not affect egg hatching but prevented molting of all prezoeae to zoeae. The concentration that killed 50% of the first stage zoeae during a 96-hr exposure (96-hr EC_{50} for death) was estimated to be 0.01 mg/liter. Few zoeae were killed in 24 hr by 82.0 mg/liter, but the 24-hr EC_{50} for death within 15 days after the exposure was estimated to be 0.015 mg/liter. The 24-hr EC_{50} for cessation of swimming, which was not always permanent, was 0.0065 mg/liter. Survival of zoeae after 25 days exposure to concentrations of 0.0001, 0.00032, 0.001, 0.0032, and 0.01 mg/liter were 83, 60, 69, 21, and 0%, respectively, and control survival was 79%. Molting was delayed at a concentration as low as 0.0001 mg/liter.

Young juvenile crabs are more sensitive to Sevin than are older juveniles or adults. The 24-hr EC_{50}'s for death or irreversible paralysis were estimated to be 0.076 and 0.35 to 0.62 mg/liter for second and ninth stage juveniles, respectively. The behavior, growth, and survival of juvenile crabs were not affected when the animals were exposed to 0.032 mg/liter of Sevin for 24 hr and then held in clean sea water for 44 days. The 24-hr and 96-hr EC_{50}'s for death or irreversible paralysis were 0.49 and 0.26 mg/liter, respectively, for adult crabs. After eating cockle clams that had just been exposed for 24 hr to 1.0, 3.2, and 10.0 mg/liter of Sevin, 22, 77, and 100% of adult crabs, respectively, were irreversibly paralyzed within 6 hr. The significance of these findings in field application of Sevin to control oyster pests and predators is discussed.

Received July 21, 1969

THIS STUDY was made to determine the effects of the insecticide Sevin, 1-naphthyl methylcarbamate, on survival and growth of the Dungeness crab, *Cancer magister*, at various stages of its life history. We chose this crab as the test animal because of its economic importance and its abundance in estuaries where it could be exposed to Sevin, which has been used to control oyster pests and predators (Butler et al., 1968). The only reported laboratory study of the effects of Sevin on *C. magister* is that of Stewart et al. (1967), who studied the acute toxicity of Sevin and its first hydrolytic product, 1-naphthol, to the late juveniles.

---

[1]Technical Paper No. 2727, Oregon Agricultural Experiment Station.

Printed in Canada (J1535)

The experiments were carried out from September 1967 to April 1969 in the Pacific Fisheries Laboratory of Oregon State University's Marine Science Center at Newport, Oregon.

MATERIALS AND METHODS

The Sevin, obtained from Miller Products Company, Portland, Oregon, was a microfine wettable powder that contained 80% active ingredient and 20% inert materials. Stock solutions were prepared by dissolving 125 mg of Sevin in 1 liter of filtered sea water. Each stock solution was stirred for 2 hr to dissolve the toxicant. Test solutions were prepared from the stock solutions by serial dilution, using filtered, sterilized sea water adjusted to 25‰ salinity with distilled water. Logarithmic series of concentrations were used. Stock and test solutions were used within 5 hr after preparation. In every experiment, each concentration was tested in duplicate or in triplicate. Each EC_{50} value (the concentration of Sevin that produced a designated effect on 50% of the test animals) was determined by straight-line graphical interpolation (American Public Health Association et al., 1965). In the 16 experiments, the criterion of toxic effect varied with the life-history stage tested (Table 1). With a few noted exceptions, the experiments were done under continuous light with standing sea water at 10 ± 1 C.

TABLE 1. Conditions of experiments and toxicity criteria for each life-history stage of the Dungeness crab tested.

Life-history stage	Expt. no.	Range of Sevin concentrations (*mg/liter*)	Exposure time (*hr*)	Postexposure observation time (*days*)	Criteria of toxic effect
Fertilized eggs and prezoeae[a]	1–3	0.0001–1.0	24	8	Prevention of hatching and molting
Zoeae	4–7	0.0032–82.0	24, 48, or 96	0	Death[b]
	8	0.0003–10.0	1, 5, 24	25	Cessation of swimming, prevention of molting, and death[b]
	9	0.0003–10.0	1, 24	8	"
	10	0.0001–0.01	25 days	0	Prevention of molting and death[b]
Juveniles					
2nd stage	11	0.0032–10.0	1, 24, 48	3	Death or paralysis[c]
3rd stage	12	0.01 and 0.032	24	44	Prevention of molting, and death or paralysis[c]
9th stage	13, 14	0.18–1.0	96	0	Death or paralysis[c]
Adults	15	0.18–1.0	96	0	"
	16	1.0–10.0[d]	24	0	"

[a]Prezoeae are those that hatched from eggs during exposure to Sevin.
[b]Cessation of heart beat.
[c]Paralysis was irreversible and was considered equivalent to death.
[d]These crabs were exposed to Sevin by feeding them cockle clams that had just been exposed for 24 hr to the indicated concentrations of Sevin.

Eggs and Prezoeae

Three experiments (Table 1) were done to determine the effects of Sevin on hatching of eggs and molting of the hatched prezoeae to zoeae. A free prezoeal stage of 5–15 min in duration is normal in the life history of *C. magister* (Buchanan and Millemann, 1969).

Ovigerous crabs caught in the ocean off the Oregon coast were held in the laboratory in 31-gal (117.8-liter) tanks containing flowing sea water at approximately 10 C. When hatching began, eggs of normal appearance with all cuticular layers intact were gently removed from the crabs and placed in 250-ml flasks. Each flask contained an average of 20 eggs and 200 ml of test solution. Eggs from one female only were used in each experiment, but eggs from different crabs were used in the three experiments. The numbers of unhatched eggs, prezoeae, and zoeae in the test vessels were recorded at the end of the 24-hr exposure periods. At this time, 117 exposed prezoeae that failed to molt to zoeae in two of the three experiments were transferred to flasks containing clean, filtered, sterilized sea water to determine if the effect of Sevin on molting of prezoeae was irreversible. The prezoeae were fed either brine shrimp larvae (*Artemia salina*) or barnacle larvae (*Balanus glandula*) on alternate days. The water in the flasks was changed at the time of feeding. The tests were terminated at 8 days and the numbers and stages of surviving larvae recorded.

Zoeae

Seven experiments (Table 1) were done to determine the effects of Sevin on survival, molting, and swimming of first stage zoeae. Experiments 4–9 involved short-term exposures (96 hr or less) to the chemical, whereas in experiment 10 the larvae were exposed for 25 days.

The larvae were progeny of crabs held in the laboratory. Those used in any one experiment all came from one female. Larvae used in experiments 4, 5, and 7 all came from the same female, and those used in the remaining experiments came from different females. At the beginning of each experiment, swimming larvae were selected randomly from the holding vessels and usually 10 were placed into a test vessel (250-ml beaker) containing 200 ml of the test solution.

Unfed larvae 1–2 days old were tested in experiments 4–6 at 17 ± 1 C and those in experiment 6 were tested also at 10 ± 1 C. In preliminary tests Buchanan (MS, 1970) found that feeding is not necessary during tests of such brief duration; 90% or more of unfed larvae survived in sea water for at least 7 days. In experiment 7, 10-day-old larvae were tested. These larvae, in groups of 50 held in 1-gal (3.8-liter) glass jars, had been fed barnacle larvae on alternate days for 10 days before they were used. The sea water was changed at the time of feeding.

In experiments 8 and 9, the zoeae were 1 and 8 days old, respectively. The 8-day-old larvae were held in groups of 50 in 1-gal (3.8-liter) glass jars and were fed brine shrimp larvae on alternate days before use. After exposure to Sevin, the larvae were transferred to beakers containing 200 ml of clean, sterilized sea water. The zoeae in each beaker were fed about 300–400 brine shrimp larvae three times a week. The water was renewed in each beaker at the time of feeding, and at this time the larvae were observed for survival, molting, and cessation of swimming.

The 2-day-old crab larvae used in experiment 10 were exposed alternately to light for 16 hr and to darkness for 8 hr during exposure to Sevin. The zoeae in each beaker were fed about 1000 brine shrimp larvae daily for the first 15 days of the experiment and three times a week thereafter; in a preliminary experiment, brine shrimp larvae were not affected in 24 hr even by 1.0 mg/liter of Sevin. The test solutions were changed at the time of feeding. The crab larvae were observed daily for survival and molting.

Juveniles

In experiment 11 (Table 1), second stage juvenile crabs, averaging 9.5 mm in carapace width, were held for 2 days in sea water at the test temperature of 14 ± 1 C and at the test salinity before transfer in groups of six to 1-gal (3.8-liter) glass jars containing 2 liters of test solution. After exposure, they were transferred to clean, standing sea water and observed for 3 days for delayed toxic effects. The animals were not fed during the experiment.

Experiment 12 (Table 1) was done to determine if 24-hr exposure to sublethal concentrations of Sevin affects survival, molting, and the behavioral interactions between exposed and unexposed

crabs held together. The crabs were third-stage juveniles with an average carapace width of 14.5 mm. Sex of the crabs was used as a marker to distinguish between exposed and unexposed animals because there is no other known marker for crabs of this size that will persist through molting. The sex of third-stage juvenile crabs can be determined by raising the abdomen and looking for oviduct openings. However, this procedure can damage the brittle abdomen. We have found that the sex of third-stage juveniles can be quickly and easily determined by the external shape of the second abdominal segment. The edges of this segment are straight in males and curved in females.

In experiment 12, the crabs were held for 5 days in sea water at 12 ± 1 C before transfer in groups of six to 1-gal (3.8-liter) glass jars containing 2 liters of test solution. Then a group of 10 crabs of each sex was removed from each exposure concentration and subdivided into two equal subgroups. Each subgroup was placed with five unexposed crabs of the opposite sex and approximately the same size into a 13.3-liter plastic container. Thus, there were eight containers each containing five exposed crabs of one sex and five unexposed crabs of the opposite sex. Two additional containers each had five male and five female crabs from one exposure concentration and two other containers each had five male and five female crabs from the other exposure concentration. There were also two control containers each with five unexposed female and five unexposed male crabs. The 14 containers were supplied with flowing, filtered, aerated sea water at 12 ± 1 C at a rate of 400 ml/min. The bottom of each container was covered with sand and broken clam shells 4 cm deep. The crabs were exposed alternately to light for 16 hr and darkness for 8 hr. They were fed chopped cockle clams (*Clinocardium nuttalli*) daily, and at that time deaths and molts were recorded. Carapace widths of the crabs were measured at the end of the experiment and compared with initial widths.

The crabs used in experiments 13 and 14 (Table 1) were probably ninth-stage juveniles. Their carapace widths ranged from 58 to 80 mm and averaged 67 mm. They were held at the test temperature of 18 ± 1 C and salinity of 25‰ for 4 days, and then placed in groups of five into 5-gal (19.0-liter) glass jars containing 15 liters of aerated test solution. The test solutions were changed daily during the 4-day experiment.

Adults

Two experiments using adult crabs were done (Table 1). The crabs used in experiment 15 were females and averaged 110 mm in carapace width. They were held at the test temperature of 11 or 18 C and a salinity of 25‰ for 4 days, and then placed in groups of three into 5-gal (19.0-liter) glass jars containing 15 liters of aerated test solution. The test solutions were changed daily during the 4-day experiment.

Experiment 16 was a secondary poisoning test (Table 1). Adult male and female crabs with an average carapace width of 100 mm were fed cockle clams that had just been exposed to different concentrations of Sevin. Clams with a mean shell length of 60 mm were exposed to Sevin in groups of 11 for 24 hr in 5-gal (19.0-liter) glass jars containing 15 liters of test solution at 13 ± 1 C. The concentrations used were 1.0, 3.2, and 10.0 mg/liter. Clams in the last concentration became paralyzed, i.e., they were unable to close the shell or retract the extended foot. After exposure, the clams were removed from their shells, rinsed in clean sea water, and weighed. Some of these clams were fed to the crabs and the others were saved for tissue analyses. A group of three crabs was placed into each of 12 13.3-liter plastic containers provided with flowing sea water of 25–32‰ salinity at 11.0 ± 1 C. The 12 containers were arranged into 4 equal subgroups. Each container in each subgroup received either three unexposed clams or three clams from one of the above exposure levels. The numbers of paralyzed or dead crabs were recorded at 6 hr and again at the end of the 24-hr experiment. The clam tissue in each container was weighed at the beginning and end of the experiment, and the amount eaten by the crabs was computed.

Tissue Analyses

Tissues from 27 clams, 9 from each of the 3 exposure groups from the above experiment, were analyzed for Sevin and free 1-naphthol, the first hydrolytic product of Sevin. Control animals for the analyses were nine unexposed clams. Clams that had been removed from their shells were

treated and analyzed as follows: (1) animals from each group were pooled and thoroughly homogenized in a blender; (2) 20 g of clam homogenate were mixed with 90 g of anhydrous sodium sulfate; (3) the mixtures were placed in a paper extraction thimble in a glass jar, and about 100 ml each of pentane and ether were added; (4) the samples were stored at 3 C until analyzed; (5) the samples were extracted and the extracts purified using a modification of the procedure of Johnson et al. (1963); and (6) the colorimetric procedure described by Karinen et al. (1967) was used for determination of Sevin and free 1-naphthol.

RESULTS

Eggs and Prezoeae

Sevin did not affect hatching of eggs. The percentages of eggs that hatched in the 24-hr exposure periods (experiments 1–3) ranged from 65 to 77 for those exposed to the highest concentration of Sevin (1.0 mg/liter) and from 53 to 76 for untreated eggs.

Of the prezoeae that hatched in the control vessels, 85–95% molted to zoeae in the 24-hr exposure period. With Sevin concentrations increasing from 0.0001 to 1.0 mg/liter the percentages of prezoeae that molted decreased from about 85 to 0, respectively (Fig. 1). The 24-hr EC_{50}'s for the three experiments were 0.006, 0.02, and 0.03 mg/liter.

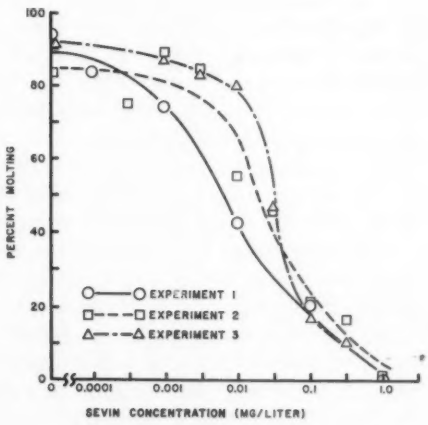

FIG. 1. Percentages of Dungeness crab prezoeae (at least 21 used for each concentration in each experiment) that molted during 24 hr exposure to various concentrations of Sevin. The prezoeae hatched from eggs that were obtained from three female crabs, one for each experiment.

Of the 117 prezoeae that did not molt, none that had been exposed to Sevin concentrations greater than 0.032 mg/liter were alive after 8 days in clean water. Only 8% of those exposed to the lower concentrations molted and survived.

Zoeae

In experiments 4–7, few larvae were killed in 48 hr even at concentrations as high as 82.0 mg/liter at 10 ± 1 C; however, the majority were not swimming at concentrations as low as 0.005 mg/liter. In the one 96-hr experiment at 10 ±

1 C, the 96-hr EC_{50} for death was 0.01 mg/liter. In the one experiment at 17 ± 1 C, the 24- and 48-hr EC_{50}'s were 0.08 and 0.005 mg/liter indicating a marked temperature effect.

In experiment 8, all larvae survived the periods of exposure to Sevin, but many ceased swimming during these times. However, after their return to clean water many resumed swimming, but thereafter increasing numbers stopped swimming as shown by the EC_{50} values for cessation of swimming (Table 2). These EC_{50} values for any observation period decreased progressively with increase of exposure time. The EC_{50}'s for death decreased progressively with increase in exposure time by day 8 of the experiment, and by day 15 approximated or equalled those for cessation of swimming. These results show that cessation of swimming at the end of 24 hr can be used to indicate the delayed lethal effects of Sevin and would be the more useful criterion in laboratory studies. By day 15, the percentages of survival of zoeae exposed to Sevin concentrations between 0.03 to 1.0 mg/liter decreased markedly as the duration of exposure to these concentrations increased (Fig. 2). In this experiment, Sevin did not affect molting of the surviving larvae. At the end of the experiment, all surviving control and exposed larvae had molted to second stage zoeae.

In experiment 9, the results were generally the same as those obtained in experiment 8. All larvae were alive at the end of the exposure periods, but on day 3 some were dead. The EC_{50}'s for death at this time for the 1- and 24-hr exposures were 0.57 and 0.024 mg/liter, respectively, on day 6 they were 0.24 and 0.019 mg/liter, and on day 8 they were 0.19 and 0.017 mg/liter. As in the previous experiment, there was an immediate effect on swimming. Again, many larvae recovered from this paralysis but some of these died later. On day 8, the EC_{50}'s for cessation of swimming were identical with those for death. Survivals of control larvae on days 6 and 8 were 93 and 77%, respectively.

In experiment 10, when larvae were exposed to Sevin for 25 days, all were alive at the end of day 1 but on day 2 some of those exposed to the two highest concentrations were dead (Fig. 3A). The 96-hr EC_{50} for death was 0.009 mg/liter, which nearly agrees with that obtained in the 96-hr experiment mentioned above. Survival of larvae at the highest Sevin concentration of 0.01 mg/liter decreased from 97% on day 3 to 10% on day 5 and 3% on day 20. On day 20, survival of larvae at the other concentrations ranged from 73 to 88%, in comparison with 83% for the controls. On day 25, the percentages of larvae surviving at Sevin concentrations of 0.0001, 0.00032, 0.001, 0.0032, and 0.01 mg/liter were 83, 60, 69, 21, and 0, respectively, and control survival was 79%. The 20- and 25-day EC_{50}'s for death were 0.005 and 0.002 mg/liter, respectively.

There was a direct relationship between Sevin concentration and delay or prevention of molting of larvae in experiment 10 (Fig. 3B). The effect on molting of larvae to second-stage zoeae was first observed on day 17. At that time, 28% of the controls had molted and were living, in comparison with 7% at the lowest Sevin concentration of 0.0001 mg/liter and none at the three

TABLE 2. EC_{50}'s (mg/liter) for cessation of swimming and for death for 1-day-old zoeae exposed for different periods to Sevin and then held in clean sea water for periods up to 24 days.[a]

Exposure time (*hr*)	Criterion of toxic effect	Days after beginning of exposure										
		≤1[b]	3	6	8	11	13	15	18	20	22	25
1	Cessation of swimming	0.056	1.7	1.7	1.6	1.6	1.4	1.2	0.56	0.26	0.21	0.21
	Death	>10.0	2.5	1.9	1.8	1.7	1.6	1.3	0.56	0.26	0.21	0.21
5	Cessation of swimming	0.018	0.78	0.73	0.55	0.52	0.22	0.13	0.12	0.11	0.032	0.024
	Death	>10.0	>10.0	2.1	1.4	0.62	0.45	0.18	0.12	0.11	0.032	0.024
24	Cessation of swimming	0.0065	0.017	0.016	0.016	0.016	0.016	0.015	0.013	0.012	0.0032	0.0032
	Death	> 1.0	0.13	0.017	0.016	0.016	0.016	0.015	0.014	0.012	0.0032	0.0032

[a]For both exposure and observation periods the salinity of the sea water was 25‰ and the temperature 10 ± 1 C. After exposure the larvae were transferred to clean sea water and fed brine shrimp larvae three times a week. Survival of control larvae on days 15 and 25 was 90 and 79%, respectively.

[b]Calculated at the ends of the exposure periods; no larvae died during these times.

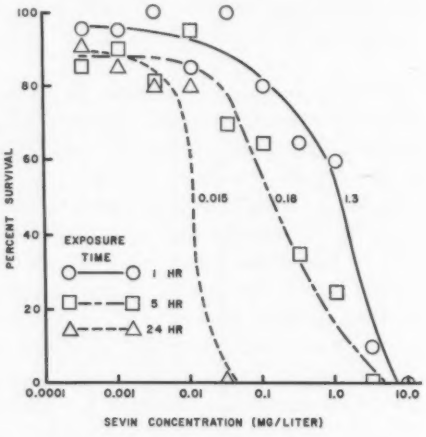

FIG. 2. Percentages of survival of zoeae on day 15 in clean sea water at 10 ± 1 C after exposure at 1 day of age to various concentrations of Sevin for three time periods (experiment 8). Twenty larvae were used for each concentration. The number beside each curve is the EC_{50} value. Survival of control larvae was 90%.

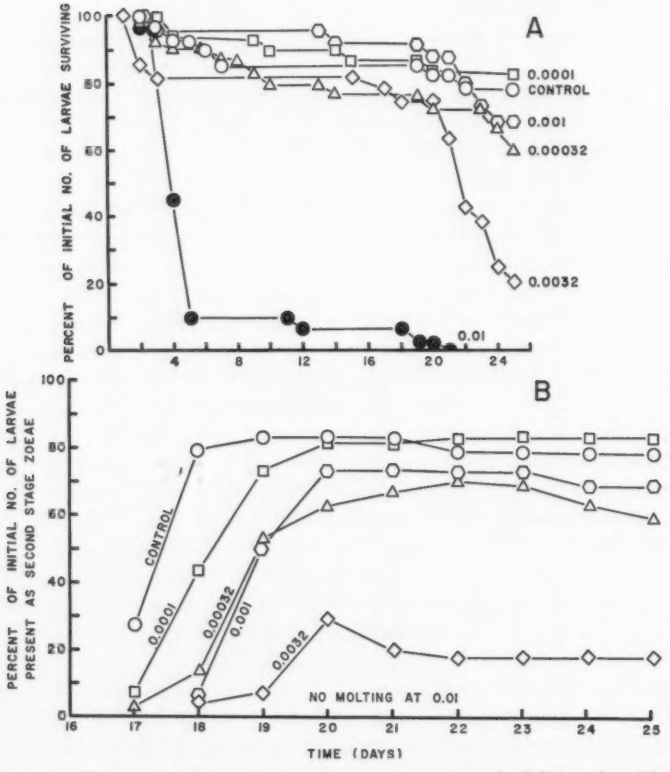

FIG. 3. Effects of different Sevin concentrations on survival (A) and molting (B) of first stage zoeae. The larvae in 3 groups of 10 were exposed to each Sevin concentration for 25 days. The number beside each curve is the toxicant concentration used.

highest concentrations. On day 18, 79% of the controls and 43% of the larvae exposed to 0.0001 mg/liter and less than 15% of those exposed to higher concentrations had molted and were still living. At the end of the experiment, however, all surviving larvae except one exposed to 0.0032 mg/liter were second-stage zoeae.

On day 3 of experiment 10, we found all of the larvae infested with a microorganism, which was either an alga or a fungus. The infestation was lost when the larvae molted. We do not know if the infestation affected survival and molting of the larvae, but at the end of the experiment 79% of the controls, all of which had been infested, had molted and were alive. This percentage was the same as that of control larvae in experiment 8, which also lasted 25 days and in which no infestation of the larvae was seen.

Juveniles

The 1-hr EC_{50} for death or paralysis of second-stage juveniles (experiment 11) was 4.3 mg/liter, but it decreased to 1.5 mg/liter within 3 days after exposure. The 24- and 48-hr EC_{50}'s for death or paralysis were 0.076 and 0.057 mg/liter, respectively, and they were the same 3 days after exposure.

Sevin did not affect growth or survival of third-stage juveniles (experiment 12). The mean percent increases in carapace width for controls and for crabs exposed to 0.01 mg/liter when the unexposed and exposed crabs were held together were 92 and 91, respectively. They were 94 and 92% for controls and crabs exposed to 0.032 mg/liter and held together. The mean percent increases for control crabs and for those exposed to 0.01 and 0.032 mg/liter of Sevin when these groups were held separately were 93, 93, and 96, respectively. Survival percentages for all controls and for all crabs exposed to 0.01 and 0.032 mg/liter were 88, 88, and 85, respectively. Those deaths that did occur were due to cannibalism, newly molted crabs having been eaten by others. Sevin apparently did not affect the normal behavioral interaction of crabs. For example, we did not observe differences in aggressiveness or in burrowing ability between control and exposed crabs.

The 24-, 48-, and 96-hr EC_{50}'s for death or paralysis of ninth-stage juveniles (experiments 13 and 14) were 0.35 and 0.62, 0.30 and 0.32, and 0.22 and 0.28 mg/liter, respectively.

Adults

Sevin was more toxic to adult crabs at 18 than at 11 C as shown in experiment 15 (Fig. 4). The 24-hr EC_{50}'s for death or paralysis at 18 and 11 C were 0.32 and 0.49 mg/liter, and the 96-hr EC_{50}'s were 0.18 and 0.26 mg/liter, respectively.

Within 6 hr in experiment 16, 22, 77, and 100% of the crabs were paralyzed after eating clams that had been exposed to 1.0, 3.2, and 10.0 mg/liter of Sevin (Table 3). One additional crab, which had eaten clams exposed to the intermediate Sevin concentration, became paralyzed after more than 6 hr.

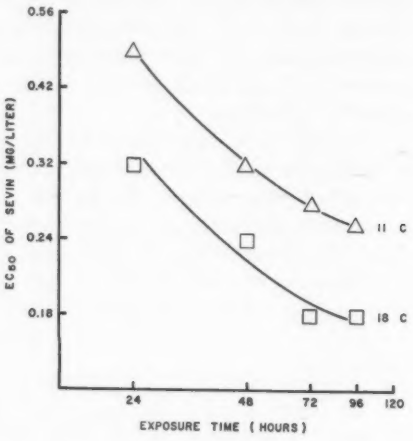

FIG. 4. EC_{50}'s of Sevin for death or paralysis of adult female crabs at 11 and 18 C.

TABLE 3. Effects on adult crabs of feeding on cockle clams previously exposed to Sevin.[a]

Sevin concentration (*mg/liter*) in water	Tissue concentration in clams (*ppm*)[b]		% survival of clams[c]	Mean wt (*g*) of clam tissues		% crabs paralyzed after:	
	Sevin plus free 1-napthol	Sevin alone		Fed	Eaten	6 hr	24 hr
0.0	–[d]	–[d]	100	14.4	14.4	0	0
1.0	3.45	–[e]	100	15.0	13.8	22	22
3.2	13.38	8.60	100	15.4	10.8	77	89
10.0	39.45	31.30	0	15.4	9.0	100	100

[a]Nine clams and nine crabs were used at each concentration. Crabs in groups of three were fed exposed clams and were observed for irreversible paralysis at 6 and 24 hr.
[b]On a wet-weight basis.
[c]At the end of the 24-hr exposure period.
[d]Unexposed tissues were analyzed as colorimetric blanks for background absorption.
[e]Sample was lost during analysis.

Control crabs remained normal during the experiment. Crabs fed clams exposed to 0.0, 1.0, 3.2, and 10.0 mg/liter of Sevin ate 14.4, 13.8, 10.8, and 9.0 g of clam tissue, respectively (Table 3).

DISCUSSION

Of the life-history stages of *C. magister* that we tested, the early larvae were the most sensitive to Sevin. For example, a Sevin concentration of 1.0 mg/liter did not affect egg hatching, but it prevented molting of all prezoeae to zoeae, and a concentration as low as 0.006 mg/liter prevented molting of 50% of the larvae. The 96-hr EC_{50}'s for death of first stage zoeae, late juveniles, and adults were 0.01, 0.25, and 0.26 mg/liter, respectively. Also, there were

delayed mortalities of zoeae that were held in clean water after their exposure to Sevin (experiment 8), whereas with the same Sevin concentrations juveniles held in clean water after their exposure were not affected (experiment 12).

Of zoeae exposed for 25 days to Sevin concentrations ranging from 0.0001 to 0.001 mg/liter, at least 60% survived and molted but molting was delayed. None of the larvae exposed to the highest concentration, 0.01 mg/liter, molted. Most of those exposed to the next highest concentration, 0.0032 mg/liter, should have molted soon after day 20 (Fig. 3B) but at this time there was a marked decrease in survival (Fig. 3A). This high mortality may have been due to an increase in larval sensitivity to Sevin associated with the molting process. However, increased mortalities of larvae that were exposed to lower concentrations, and that had molted earlier, were observed between days 19 and 25. Therefore, molting cannot be said definitely to have been a cause of the greater mortality at the 0.0032 mg/liter concentration.

Young juvenile crabs are more sensitive to Sevin than are older juveniles or adults. The 24-hr EC_{50}'s for death of second-stage juveniles, ninth-stage juveniles, and adults were 0.076, 0.35–0.62, and 0.49 mg/liter, respectively. Our 24-hr EC_{50}'s for ninth-stage juveniles agree with those of Stewart et al. (1967), who reported 24-hr EC_{50}'s ranging from 0.55 to 0.70 mg/liter for the late juveniles.

Post-larval Dungeness crabs have about the same sensitivity to Sevin as other species of crabs. The 24-hr EC_{50}'s for death or paralysis of small stone crabs (scientific name not given) was 1.0 ppm (Butler, 1962); for juvenile blue crabs, *Callinectes sapidus*, 0.55 ppm (Butler, 1963); and for adult shore crabs, *Hemigrapsus oregonensis*, 0.06–1.05 mg/liter (Stewart et al., 1967). The only other published study of the effects of Sevin on crabs known to us is that of Andrews et al. (1968), who reported that a concentration of 10.0 mg/liter eliminated parasitic pea crabs, *Pinnotheres ostreum*, from live oysters in 12 hr.

Our finding that adult crabs can be killed within 6 hr after feeding on cockle clams previously exposed to Sevin may explain in part findings by Snow and Stewart (1963). They observed many paralyzed cockle clams on an oyster ground in Oregon that had been treated 30 min previously with Sevin to control burrowing ghost and mud shrimps. A day later they found a large number of dead Dungeness crabs on the grounds. They suggested that the crabs may have moved on to the treated area during flood tide and been killed either by direct contact with the chemical or by feeding on paralyzed clams.

Butler et al. (1968) suggested, on the basis of their results and those of Karinen et al. (1967), that the best time for treatment of estuaries with Sevin to control oyster pests would be the period when maximum water temperatures occur. The insecticide at this time would be more rapidly degraded and perhaps be accumulated by clams in minimal amounts. Our results suggest that minimal damage to the Dungeness crab population from field application of Sevin would occur after the larvae had molted into juveniles. This larval molt in nature occurs predominantly in June in central California (Poole, 1965), from

April to July in Oregon (Waldron, 1958), and in September in British Columbia (Butler, 1961).

ACKNOWLEDGMENTS

Grateful acknowledgment is expressed to the following individuals of Oregon State University: Messrs Donald Bennett, Jerry Butler, and Dennis Wilson for technical assistance; Mr John Lamberton for the tissue analyses; and Dr Peter Doudoroff for advice given during this study.

This study was supported by Public Health Service research grant CC 00303, from the National Communicable Disease Center, Atlanta, Georgia.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1965. Standard methods for the examination of water and wastewater. 12th ed. APHA, Inc., N.Y. 769 p.

Andrews, J. D., D. Turgeon, and M. Hreha. 1968. Removal of pea crabs from live oysters by using Sevin®. Veliger 11: 141–143.

Buchanan, D. V. MS, 1970. Effects of the insecticide Sevin on the Dungeness crab, *Cancer magister* Dana. M.Sc. Thesis. Oregon State University, Corvallis, Oreg.

Buchanan, D. V., and R. E. Millemann. 1969. The prezoeal stage of the Dungeness crab, *Cancer magister* Dana. Biol. Bull. 137: 250–255.

Butler, J. A., R. E. Millemann, and N. E. Stewart. 1968. Effects of the insecticide Sevin on survival and growth of the cockle clam *Clinocardium nuttalli*. J. Fish. Res. Bd. Canada 25: 1621–1635.

Butler, P. A. 1962. Effects on commercial fisheries. *In* Effects of pesticides on fish and wildlife: a review of investigations during 1960. U.S. Fish Wildlife Serv. Circ. 143: 20–24. 1963. Commercial fisheries investigations. *In* Pesticide–wildlife studies: a review of Fish and Wildlife Service investigations during 1961 and 1962. U.S. Fish Wildlife Serv. Circ. 167: 11–25.

Butler, T. H. 1961. Growth and age determination of the Pacific edible crab *Cancer magister* Dana. J. Fish. Res. Bd. Canada 18: 873–891.

Johnson, D. P., F. E. Critchfield, and B. W. Arthur. 1963. Determination of Sevin insecticide and its metabolites in poultry tissues and eggs. J. Agr. Food Chem. 11: 77–80.

Karinen, J. F., J. G. Lamberton, N. E. Stewart, and L. C. Terriere. 1967. Persistence of carbaryl in the marine estuarine environment. Chemical and biological stability in aquarium systems. J. Agr. Food Chem. 15: 148–156.

Poole, R. L. 1965. Preliminary results of the age and growth study of the market crab (*Cancer magister*) in California: The age and growth of *Cancer magister* in Bodega Bay. *In* Proc. Symp. Crustacea. Part II. Marine Biol. Ass. India. p. 553–567.

Snow, C. D., and N. E. Stewart. 1963. Treatment of Tillamook Bay oyster beds with MGS-90 (Sevin). Inform. Rep. Fish. Comm. Oregon, Portland, Oreg. 9 p.

Stewart, N. E., R. E. Millemann, and W. P. Breese. 1967. Acute toxicity of the insecticide Sevin® and its hydrolytic product 1-naphthol to some marine organisms. Trans. Amer. Fish. Soc. 96: 25–30.

Waldron, K. D. 1958. The fishery and biology of the Dungeness crab (*Cancer magister* Dana) in Oregon waters. Fish. Comm. Oregon, Contrib. 24, Portland, Oregon. 43 p.

Growth and Vertical Distribution of the Glacier Lanternfish, *Benthosema glaciale*, in the Northwestern Atlantic

R. G. HALLIDAY

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

HALLIDAY, R. G. 1970. Growth and vertical distribution of the glacier lanternfish, *Benthosema glaciale*, in the northwestern Atlantic. J. Fish. Res. Bd. Canada 27: 105–116.

Benthosema glaciale (Reinhardt, 1837) collected off Nova Scotia can be aged using otoliths, one transparent otolith ring being laid down each year. Maximum age attained is at least 4½ years and maximum length is 68 mm. There is no sexual dimorphism in growth. Part of the population spawns for the first time at age 2 and all at subsequent ages.

In the northwestern Atlantic, *B. glaciale* is abundant in water with temperatures of 4–16 C and is found in temperatures higher than 18 C. Daylight depth range is at least 80–290 fath, the centre of distribution being below 250 fath. At night they are caught from the surface to at least 275 fath with the centre of distribution between 25 and 50 fath, indicating a diurnal vertical migration of over 200 fath for at least part of the population. Older, larger fish are found deeper than young fish at night.

Received October 14, 1969

INTRODUCTION

BENTHOSEMA GLACIALE (Reinhardt, 1837), a low arctic-temperate species (as defined by Ekman, 1953), is the most common member of the Myctophidae in the North Atlantic north of about 40°N and in west Greenland and Norwegian Seas. It is found in the Slope Water region north and west of the Gulf Stream system as far south as Cape Hatteras (Goode and Bean, 1896), around Newfoundland (Leim and Scott, 1966), and in the Davis Strait and southern Baffin Bay to 70°40′N (Jensen, 1948). Johnsen (1922, 1944) records its capture in numerous Norwegian fjords and in the Norwegian Sea to 80°N off Spitzbergen. A subspecies is common in the western Mediterranean (Tåning, 1918). There are also isolated records from the Cape Verde Islands (Norman, 1930) and Point Barrow, Alaska (Wilimovsky, 1954).

Although lanternfishes are one of the most abundant groups of mesopelagic fishes both in numbers of species and individuals, few life-history and ecological studies have been published. Exceptions are the works of Ogawa (1961), Odate and Ogawa (1961), and Odate (1966) on *Myctophum affine* (Lütken) off eastern Japan and that of Bolin (1956) on *Lampanyctus leucopsarus* (Eigenmann and Eigenmann) off central California. Aspects of the life history of *B. glaciale* are discussed by Tåning (1918) and Johnsen (1922, 1944). The present study establishes an ageing technique for this species, and discusses

Printed in Canada (J1620)

growth, maturity, and vertical distribution in relation to depth and temperature in the northwestern Atlantic.

MATERIAL AND METHODS

Between May 1967 and January 1969, 4350 specimens of *B. glaciale* were collected by vessels of the Fisheries Research Board of Canada (FRB) over the continental slope (bottom depths 100–750 fath) off central Nova Scotia. Also available to the author were 41,250 specimens from cruise 13 of the Woods Hole Oceanographic Institution's vessel *Atlantis II*. This cruise covered an extensive area of the northwestern Atlantic between 38–48°N and 26–70°W from September 1 to October 5, 1964 (Fig. 1).

On FRB cruises, a 6-ft Isaacs-Kidd midwater trawl without closing devices was the only collecting gear. Depth fished was estimated from wire angle and wire out. Temperature data were from bathythermograph traces made after selected tows. Most *Atlantis II* tows were made with a 10-ft Isaacs-Kidd midwater trawl, and a few with a Marinovich midwater trawl — a net about 7 times larger than the 10-ft Isaacs-Kidd. Neither had closing devices. Net depth and temperature records were obtained using Benthos Co. time-depth and time-temperature recorders attached to the gear.

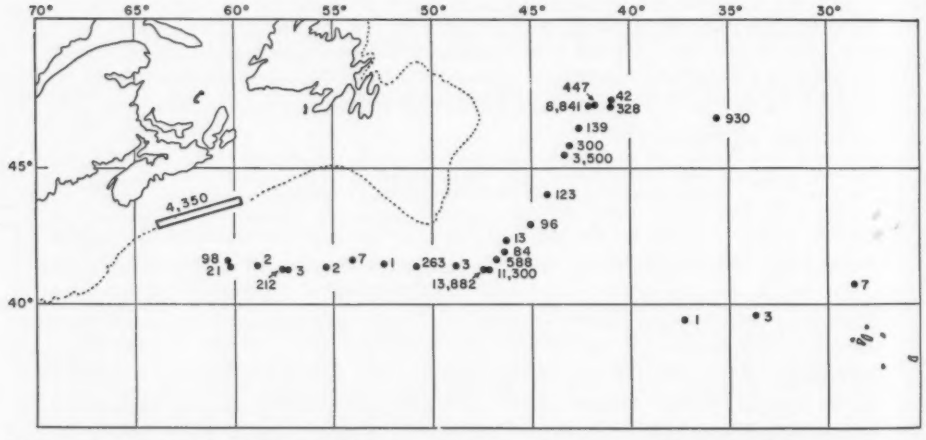

FIG. 1. Geographical distribution of *Benthosema glaciale* catches. Dots — *Atlantis II*, September–October 1964. Box — FRB vessels, May 1967–January 1969. Number of specimens indicated for each station.

The method of measuring depth fished on FRB cruises, using wire angle and wire out to calculate net depth, does not give high accuracy. However, the results should be reliable when grouped into wide depth zones of 25 and 50 fath as in Tables 3 and 4. As closing devices were not used, contamination of the catch during setting out or hauling in of the net, by fishes living at lesser depths, was possible. This was minimised on the *Atlantis II* cruise by taking long tows of 1½–4¾ hr duration. It may have had more effect on the deeper FRB tows that lasted only 30 min. A further possible source of error is that specimens, stuck in the meshes of the net, can be carried over into succeeding tows. Although the nets were thoroughly washed after each tow, this may have occurred on occasion.

Those specimens whose otoliths were used for ageing were preserved in 70% alcohol, the others in formalin. Otoliths immersed in absolute alcohol were viewed by binocular microscope

against a black background using reflected light. Age was calculated from January 1. All body measurements were of standard length, i.e., excluding caudal fin.

AGE DETERMINATION

The modes of length-frequency distributions have often been used to deduce the age of fishes (Petersen's Method), as have the rings on bony structures such as scales and otoliths (see Le Cren (1947) for review). Otoliths of *B. glaciale* from off Nova Scotia clearly showed alternate opaque and transparent rings (Fig. 2). When the catch in any one month was divided into groups on the basis of the number of transparent rings, each of these groups was found to correspond to a peak in the length-frequency distribution (Fig. 3).

Two post-larvae, 9.5 mm and 11.5 mm long, were taken in July 1967. Fully metamorphosed specimens down to 11 mm were caught in July 1968. Thus, metamorphosis probably occurs at a length of 11–12 mm and those specimens 10–25 mm long forming the mode at 16.5 mm in the July 1968 sample, i.e., with no transparent otolith rings, were young-of-the-year fish. This mode at 16.5 mm can be followed through the length-frequency distributions for subsequent months (Fig. 4). It corresponds to that at 22.5 mm in October 1968 and that at 24.5 mm in January 1969. Assuming growth rate is similar in different years, as appears to be the case when the July modes for 1967 and 1968 are compared, this peak must correspond to that at 30.5 mm in May 1967 and to the one at 34.5 mm in July of both 1967 and 1968. This peak is composed of fish with one transparent otolith ring. Similarly, when this mode is followed through the length-frequency distributions, it is found to correspond, 1 year later, to that at 44.5 mm composed of fish with two transparent otolith rings. Thus, the number of transparent otolith rings is indicative of the age of the fish.

GROWTH

No sexual dimorphism in growth rate was indicated by calculations of mean length at age by sex (Table 1). In July, soon after metamorphosis, juveniles had a mean length of 17.3 mm (Table 2). By the time they were 1 year old in January and February, they had a mean length of 24.6 mm. Increments in the 2nd and 3rd years of life were approximately 13 mm and 9 mm respectively, giving a mean length of 37.5 mm at age 2 and 46.3 mm at age 3. The oldest fish aged using otoliths were 4 years and several months old measuring 52.5 mm and 58.5 mm, taken in July 1968. The largest specimen from FRB cruises was 67 mm caught in July 1967. Unfortunately this month's sample, which contained almost all the fish over 60 mm taken during the sampling programme, was preserved in formalin and was later found impossible to age. The length-frequency distribution (Fig. 4) suggests that some specimens may have been over 5 years old.

Atlantis II catches in September 1964 were composed very largely of 0-group fish. Those catches east of Flemish Cap that contained moderate numbers of large fish divided into two size groups, one composed mainly of fish

between 18 and 23 mm and the other of fish between 40 and 60 mm. The first corresponds to the October 1968 peak for 0-group fish at 18–25 mm from off Nova Scotia. Otolith examination confirms this conclusion. Those *Atlantis II* specimens 40–60 mm appear from both their length-frequency distribution and their otoliths to be 2-, 3-, and 4-year-old fish. Otolith reading from older fish proved difficult, however. Rings were less clear and less regular than in the otoliths of Nova Scotia fish, and formalin preservation, although it had not made the otoliths completely opaque, had made their edges cloudy. One-year-old fish were virtually absent from the *Atlantis II* catches.

All the fish in the February 1968 to January 1969 samples were fitted to the von Bertalanffy growth equation:

$$l_t = L_\infty \ (1 - \exp\ [-K(t - t_0)]),$$

where l_t is the length at age t, L_∞ the theoretical maximum length, K the rate at which this length is approached, and t_0 a constant. The method used was that of Allen (1966), which gives equal weight to all observations. Age was expressed in fractions of years calculated arbitrarily from April 1. The resulting equation was:

$$l_t = 68.28 \ (1 - \exp\ [-0.36 \ (t + 0.49)])$$

with standard errors of $K = \pm 0.0209$; $t_0 = \pm 0.0262$; $L_\infty = \pm 2.0262$. The value of 68 mm for L_∞ is close to the length of the largest specimen recorded on FRB cruises (67 mm) and identical to the largest caught on the cruise of the *Atlantis II*, suggesting that most of the size and age range of the population has been sampled.

SIZE AND AGE AT MATURITY

Ripening females, i.e., those with enlarged ovaries containing yolky eggs, were found in February 1968 and January 1969 samples. In the February 1968 sample, which contained 85 fish, one 2+ female of 33 mm and one 3+ female of 52 mm were ripening. The 2+ female was the second smallest of 16 females of that age (length range 27–40 mm). All the other 2+ females were either maturing or mature, i.e., ovaries were conspicuous structures extending $\frac{1}{3}$–$\frac{1}{2}$ the length of the body cavity but without developing eggs. The 3+ female was one of two taken in this sample. All 1+ fish were immature. In January 1969, all 3+ (11 fish) and 4+ females (2 fish) were ripening. Of forty-six 2+ females, 30% were ripening. All ripening females were larger than 37 mm and all those not ripening were smaller than 40 mm. All 1+ fish were immature except females larger than 26 mm, which were maturing.

Thus, some but possibly not all of the female population spawned for the first time when 2 years old.

Males, like females, were mature in their second year of life, the testes differentiating into bulbous anterior and wide tubular posterior parts. Ripening males could not be distinguished, however.

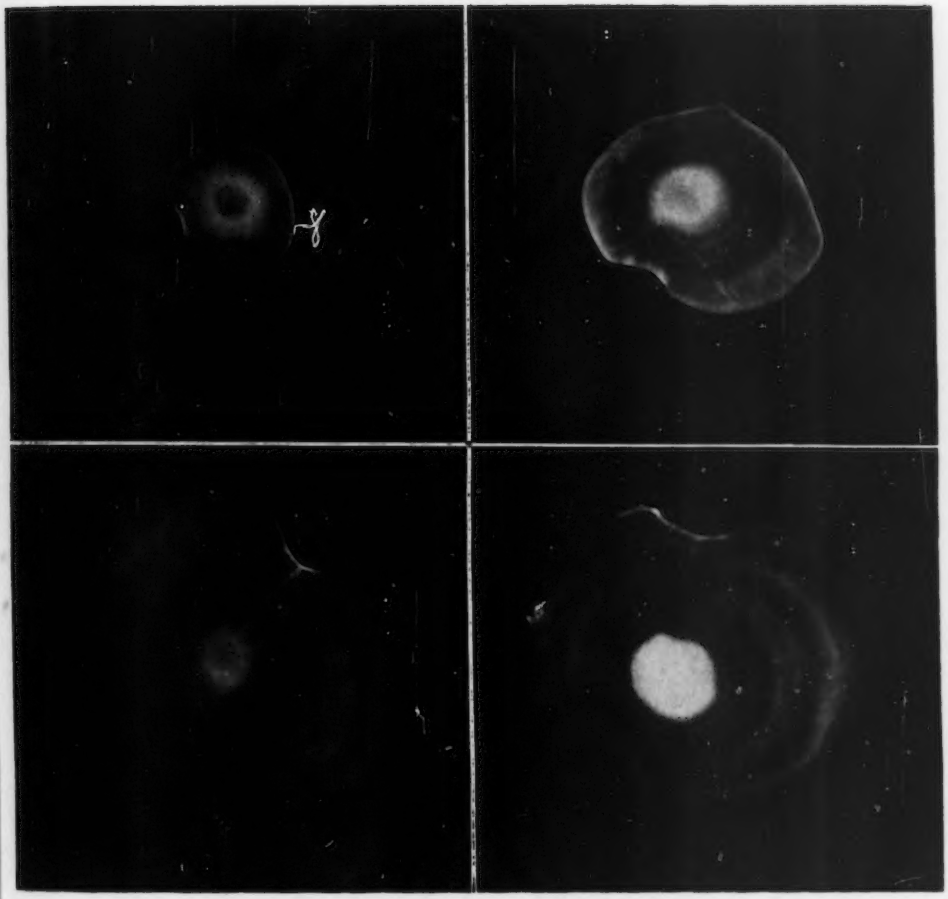

FIG. 2. Otoliths of *Benthosema glaciale* ×32. Top left: 0 year old. No transparent rings. Top right: 1 year old. 1 transparent ring, opaque edge. Bottom left: 3 years old. 3 transparent rings, opaque edge. Bottom right: 4 years old. 4 transparent rings, opaque edge.

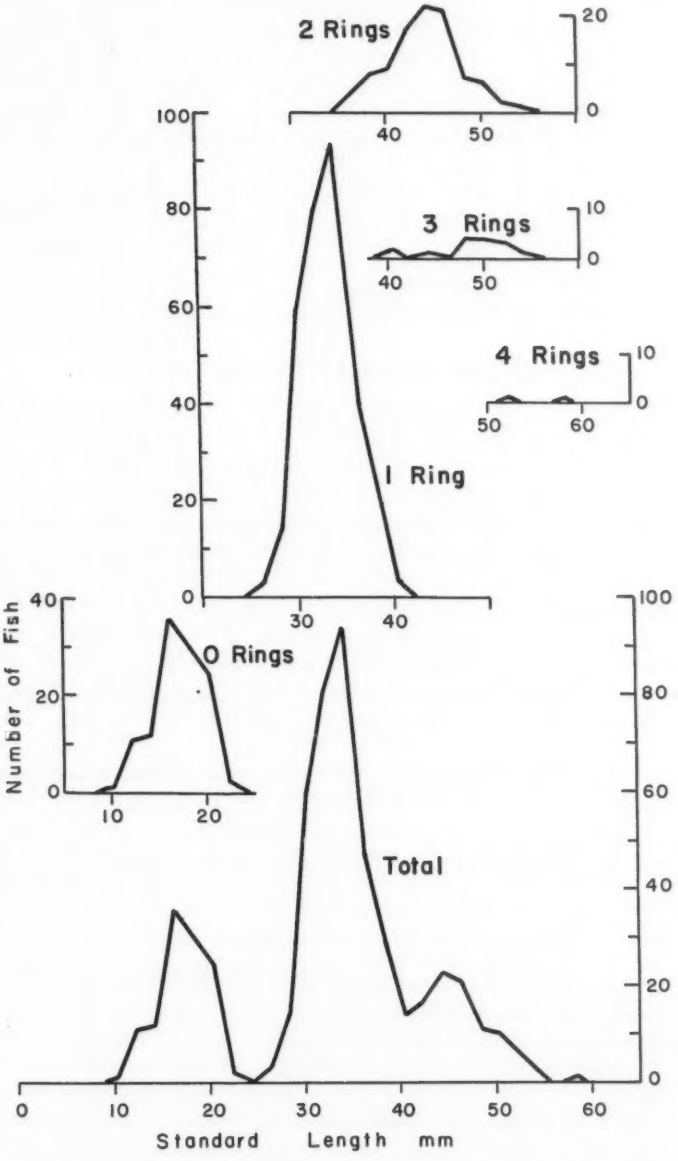

FIG. 3. Relationships of length-frequency modes in July 1968 catch of *Benthosema glaciale* to groups of fish with 0–4 transparent otolith rings.

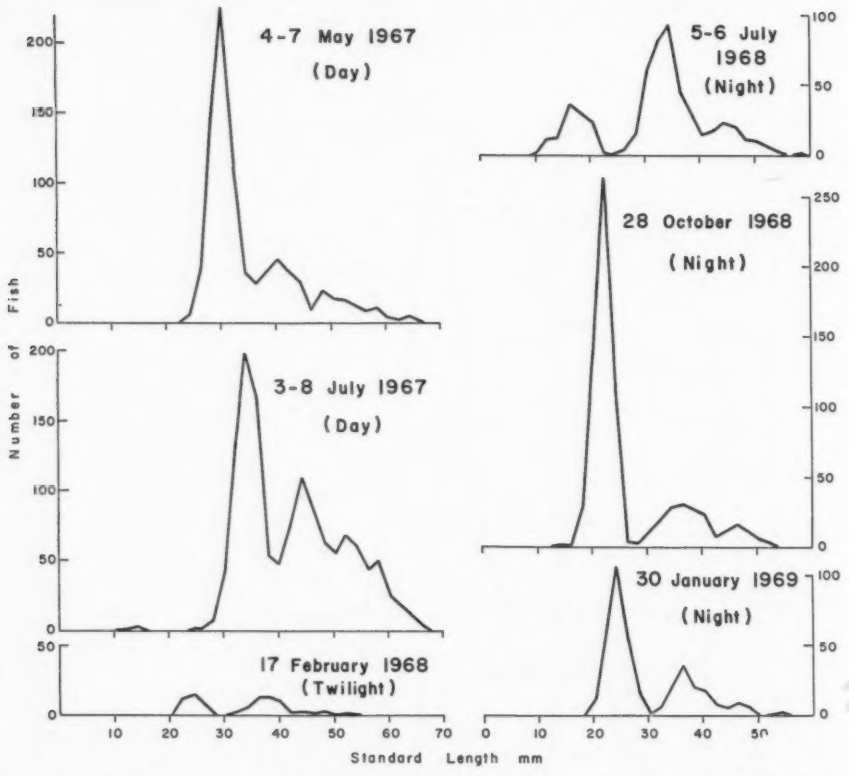

FIG. 4. Length-frequency distributions of *Benthosema glaciale* catches south of Nova Scotia, May 1967–January 1969.

TABLE 1. Mean length at age of *Benthosema glaciale* south of Nova Scotia for the sexes separately. Number of fish in parentheses. M = males; F = females. Lengths in mm.

Year-class:	1967		1966		1965	
Sex:	M	F	M	F	M	F
Feb. 1968	24.4 (16)	23.9 (18)	38.1 (29)	36.1 (16)	46.5 (4)	46.0 (2)
July 1968	33.5 (156)	33.5 (162)	44.5 (46)	44.1 (51)	45.9 (7)	51.5 (8)
Oct. 1968	36.6 (55)	36.6 (87)	46.5 (17)	47.2 (30)	–	52.5 (1)
Jan. 1969	37.5 (63)	37.6 (46)	47.3 (8)	45.6 (11)	–	54.5 (2)

TABLE 2. Mean length at age of *Benthosema glaciale* south of Nova Scotia. Number of fish in parentheses. Lengths in mm.

Age:	0	1	2	3	4
Feb. 1968	–	24.1 (34)	37.4 (45)	46.3 (6)	–
July 1968	17.3 (116)	33.5 (318)	44.3 (97)	48.9 (15)	55.5 (2)
Oct. 1968	22.2 (559)	36.6 (142)	46.9 (47)	52.5 (1)	–
Jan. 1969	–	24.6 (245)	37.5 (109)	46.3 (19)	54.5 (2)

DISTRIBUTION IN RELATION TO TEMPERATURE

Twenty-three daylight tows were made by the *Atlantis II* in waters ranging in temperature from 1.7 to 18.9 C. All catches of *B. glaciale* except one were in tows through waters with temperatures between 1.7–14.4 C. The exception was one tow between 12.8–18.9 C that took 42 specimens.

Twenty-eight night tows made on this cruise were through waters of 5–25 C, the temperature range of individual tows being extremely wide — sometimes as much as 17 C. The four tows that caught over 1000 specimens were all made in part through water colder than 15.6 C. However, six tows in water warmer than 18 C caught *B. glaciale* and 13 specimens were taken in water 21.5–24 C. This latter record of only 13 fish, taken immediately after tows in which large numbers were caught, could have resulted from contamination and cannot be considered as establishing the occurrence of *B. glaciale* in water over 21 C.

Fifty-five daylight tows were made south of Nova Scotia in May and July 1967 by FRB vessels. All specimens were taken in water less than 8.5 C and most between 5–6 C. Fifteen night tows in July and October 1968 and January 1969 caught most specimens between 4–9.5 C. A few were taken at 12.5 C near the surface.

DISTRIBUTION IN RELATION TO DEPTH

Atlantis II made daylight tows between 125–385 fath net depth, the depth fished being known accurately from time-depth recorder records. *Benthosema glaciale* was caught in both the shallowest and deepest tows, the largest catch being 588 fish taken at 220–225 fath. The deepest tow between 285–385 fath caught 96 specimens. Night tows were made in depths from 8 to 275 fath and *B. glaciale* was again caught at all depths. Large catches were made at 16–19 fath (8841 fish), 25 fath (3500 fish), 30–40 fath (13,882) and 42–82 fath (11,300 fish). Largest catch in six tows below these depths was 447 fish. 0-Group fish, which predominated in the catches, were taken in both the shallowest and deepest day and night tows.

Off Nova Scotia in May and July 1967, the greatest catch per hour of *B. glaciale* during daylight was below 250 fath (Table 3). A postlarval specimen and an adult taken at approximately 80 fath constitute the shallowest records. The adult specimen could not have been carried over from previous, deeper tows as it was the first specimen taken on FRB cruises and was taken in the first tow over the edge of the continental shelf. The greatest catch per hour at night in July and October 1968 and January 1969 lay between 25 and 50 fath (Table 3). Very few specimens were taken at the surface. A substantial part of the population must, therefore, undertake a diurnal vertical migration of over 200 fath.

TABLE 3. Distribution of fishing effort and catches of *Benthosema glaciale* in day and night tows south of Nova Scotia.

	Day				Night			
Depth (*fath*)	No. of tows	Hr fished	Catch	Catch/ hr	No. of tows	Hr fished	Catch	Catch/ hr
Surface	–	–	–	–	3	1.00	14	14.0
5–25	1	0.5	–	–	3	1.25	164	131.2
25–50	2	1.0	–	–	3	1.25	729	583.2
50–100	5	2.5	2	0.8	5	2.00	684	342.0
100–150	18	9.0	68	7.6	2	1.00	84	84.0
150–200	21	10.5	1137	108.3	–	–	–	–
200–250	7	3.5	1025	292.9	–	–	–	–
250–300	1	0.5	210	420.0	–	–	–	–
Total	55	27.5	2442	–	15	6.5	1675	–

The size composition of daylight catches did not vary regularly with depth, mean length for each depth zone being:

	May 1967	July 1967
100–150 fath	43.4 mm	38.9 mm
150–200	35.3	38.9
200–250	–	44.7
250–300	–	39.7

At night, mean length increased with depth of capture (Table 4). This reflected a change in age composition of the catches, the proportion of fish less than 1 year old (1968 year-class) decreasing, and that of the 1967 and earlier year-classes increasing, with increasing depth. Few fish greater than 55 mm were taken at night in contrast to day catches (Fig. 3), indicating that the largest specimens do not normally migrate above 150 fath (the lower limit of sampling) at night.

TABLE 4. Percentage year-class composition of nighttime catches of *Benthosema glaciale* by depth intervals, and mean length at each depth.

Date:	July 1968					Oct. 1968			Jan. 1969		
Depth (*fath*):	Surface	5–25	25–50	50–100	100–150	5–25	25–50	50–100	25–50	50–100	100–150
1968 year-class	83	46	12	2	–	100	82	66	83	61	50
1967 year-class	17	52	56	80	71	–	17	22	16	33	37
1966 and earlier year-classes	–	2	32	18	29	–	1	12	1	6	13
Mean length (*mm*)	18.7	26.3	35.7	35.2	37.4	25.0	24.6	28.4	26.5	30.1	32.8
No. of fish	12	163	246	113	14	1	377	371	106	199	70

DISCUSSION

Present records indicate that the daytime distribution of *Benthosema glaciale* in the western North Atlantic ranges from at least 80 to 290 fath. The centre of distribution is below 250 fath and the lower limit of distribution probably greater than 300 fath. Holt and Byrne (1911) conclude that the normal habitat is in depths less than 400 fath. Johnsen (1922) notes that even specimens less than 1 year old live in depths as great as 300–350 fath. Although records of capture at the surface are not infrequent (e.g., Holt and Byrne, 1911; present records) the centre of nighttime distribution lies between 25 and 50 fath. These are average figures for day and night distribution from observations made under a wide variety of light conditions. Light intensity is probably the principal environmental factor influencing vertical distribution. Thus, the distribution at any particular time may be affected by, for example, amount of cloud cover or phase of the moon. Unfortunately, present data are inadequate to confirm such variation.

Benthosema glaciale has a wide temperature tolerance being abundant in water temperatures of 4–16 C and capable of withstanding temperatures at least as high as 18 C. The southern limits of distribution in the Atlantic and the eastern limits in the Mediterranean correspond closely to the 15 C isotherm of average annual temperature at 200 m (109 fath) (Schroeder, 1963). However, occasional specimens have been taken between the 16 and 17 C isotherms (*Atlantis II* 39°25′N, 36°56′W — 1 specimen; Bolin, 1959). Temperatures as low as 0 C are also within the tolerance limits of the species. Fowler (cited by Holt and Byrne, 1911) took specimens at temperatures of 0–0.5 C in the Faeroe Channel. Also, it has been recorded from north of 70°N off the west Greenland coast (Jensen, 1948) and from Ungava Bay (Dunbar and Hildebrand, 1952) — both areas with an average annual temperature at 200 m (109 fath) less than 1 C.

Spawning occurs in early spring, ripening fish being caught in January and February and post-larvae occurring in May and July. This agrees with the results from west of Ireland of Holt and Byrne (1911) who caught larvae mainly in May, also in July and August, but not in February. Metamorphosis apparently occurs in June and July at lengths of 11–12 mm, closely similar to the length at metamorphosis of 10–11 mm found by Tåning (1918) in the Mediterranean. Part of the population spawns at age 2 and all at 3 and subsequent ages. The conclusion of Johnsen (1922) that members of this species spawn only once is not supported by the present study.

Off Nova Scotia and probably throughout the western Atlantic *B. glaciale* lives to at least 4½ years attaining a maximum length of about 68 mm. Thus, Johnsen's (1922) conclusion that 68 mm is the maximum size for this species in the temperate regions of the North Atlantic is confirmed. His deduction from length-frequency distributions based on small numbers of fish, that the maximum lifespan is 2½ years, is not substantiated, however. Greater maximum sizes are attained in more northern areas. Off the west coast of Greenland a specimen 84 mm is recorded (Jensen, 1948) and Johnsen (1944) records one

96 mm from Norwegian fjords near Stavanger. It is likely that fish from these areas also have a longer lifespan. The Mediterranean race has a maximum size of about 47 mm and a lifespan deduced from length-frequency distributions of 1½ years (Tåning, 1918). The modes of Tåning's summer sample length-frequency distribution at about 14 mm and 34 mm correspond closely to those at 16.5 mm and 34.5 mm from the July samples south of Nova Scotia. Thus, growth rate in the two areas is probably similar. It is probable also that the Mediterranean specimens 40–47 mm correspond to those from off Nova Scotia that are 2½ years old with a modal length of 44.5 mm.

Two other myctophids have been the subject of age and growth studies, *Lampanyctus leucopsarus* (Eigenmann and Eigenmann) off central California (Bolin, 1956) and *Myctophum affine* (Lütken) east of Japan (Odate, 1966). Both have a faster growth rate and shorter lifespan than *B. glaciale* off Nova Scotia. The maximum recorded size of both species is 78 mm attained in 4 and 3 years respectively.

ACKNOWLEDGMENTS

I am grateful to Dr J. S. Scott for collection of some of the material and to Drs R. L. Haedrich, A. C. Kohler, and W. B. Scott for their helpful criticism of the manuscript. Dr Haedrich provided me with the *Atlantis II* material. *Atlantis II* cruise 13 was conducted as part of National Science Foundation Grant GB-543 to the Woods Hole Oceanographic Institution, Richard H. Backus, principal investigator. The initial stages of this study were financed in part by the National Research Council of Canada.

REFERENCES

ALLEN, K. R. 1966. A method of fitting growth curves of the von Bertalanffy type to observed data. J. Fish. Res. Bd. Canada 23: 163–179.

BOLIN, R. L. 1956. Age and growth of the myctophid fish *Lampanyctus leucopsarus* (Eigenman and Eigenmann). Proc. XIV Int. Congr. Zool., Copenhagen 1953: 541.

1959. Iniomi. Myctophidae. From the "Michael Sars" North Atlantic deep-sea expedition 1910. Rep. Sci. Result. Michael Sars N. Atl. Deep Sea Exped. 1910, 4(Part II): 1–45.

DUNBAR, M. J., AND H. H. HILDEBRAND. 1952. Contribution to the study of the fishes of Ungava Bay. J. Fish. Res. Bd. Canada 9: 83–128.

EKMAN, S. 1953. Zoogeography of the sea. Sidgwick and Jackson, London. 417 p.

GOODE, G. B., AND T. H. BEAN. 1896. Oceanic ichthyology. U.S. Nat. Mus. Spec. Bull. 2: 1–529, 123 pl.

HOLT, E. W. L., AND L. W. BYRNE. 1911. Fifth report on the fishes of the Irish Atlantic slope. Fishes of the genus *Scopelus*. Sci. Invest. Fish. Br. Ireland 1910, 6(1911): 1–33, 1 pl.

JENSEN, AD. S. 1948. Contributions to the ichthyofauna of Greenland, 8–24. Skr. Udgivet Univ. Zool. Mus. Kobenhavn 9: 1–182, 4 pl.

JOHNSEN, S. 1922. Remarks on the distribution and the biology of *Myctophum glaciale* (Reinh.). Bergens Mus. Årb. 1921–22(6): 1–50, 1 pl.

1944. Studies on variation in fish in North-European waters. I. Variation in size. Bergens Mus. Årb. 1944(4): 1–129.

LE CREN, E. D. 1947. The determination of the age and growth of the perch (*Perca fluviatilis*) from the opercular bone. J. Anim. Ecol. 16: 188–204.

LEIM, A. H., AND W. B. SCOTT. 1966. Fishes of the Atlantic coast of Canada. Fish. Res. Board Can. Bull. 155. 485 p.

NORMAN, J. R. 1930. Oceanic fishes and flatfishes collected in 1925–1927. Discovery Rep. 2: 261–370.

ODATE, S. 1966. [Studies on the fishes of the family Myctophidae in the northeastern area of the Pacific coast of Japan. III. Determination of the age and growth of the susuki-hadaka lanternfish, *Myctophum affine* (Lütken).] Bull. Tohoku Reg. Fish. Res. Lab. 26: 35–43. (Fish. Res. Board Can. Transl. Ser. No. 850, 1967.)

ODATE, S., AND T. OGAWA. 1961. [Studies on the fishes of the family Myctophidae in the northeastern area of the Pacific coast of Japan. Part II. The susuki-hadaka lanternfish, *Myctophum affine* Temminck et Schlegel.] Bull. Tohoku Reg. Fish. Res. Lab. 19: 90–97. (Fish. Res. Board Can. Transl. Ser. No. 849, 1967.)

OGAWA, T. 1961. [Studies on the fishes of the family Myctophidae in the northeastern area of the Pacific coast of Japan. Part I. Species and distribution.] Bull. Tohoku Reg. Fish. Res. Lab. 19: 81–89. (Fish. Res. Board Can. Transl. Ser. No. 848, 1967.)

SCHROEDER, E. H. 1963. North Atlantic temperatures at a depth of 200 metres. Ser. Atlas Mar. Environ. Amer. Geogr. Soc. Folio 2.

TÅNING, A. V. 1918. *Myctophum glaciale* (Reinhardt). *In* Mediterranean Scopelidae (*Saurus, Aulopus, Chlorophthalmus* and *Myctophum*). Rep. Dan. Oceanogr. Exped. Mediter. 2. Biology A7: 31–45.

WILIMOVSKY, N. J. 1954. List of fishes of Alaska. Stanford Ichthyol. Bull. 4: 279–294.

Deoxyribonucleic Acid Polymerase of Trout Liver Nuclei[1]

H. L. A. TARR AND LINDA GARDNER

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

TARR, H. L. A., AND L. GARDNER. 1970. Deoxyribonucleic acid polymerase of trout liver nuclei. J. Fish. Res. Bd. Canada 27: 117–123.

The DNA polymerase of intact trout liver nuclei behaved similarly to that present in mammalian tissues in its mandatory requirement for four deoxynucleoside triphosphates, Mg^{2+}, and DNA primer. It was inhibited by DNase and pyrophosphate in comparatively low concentrations and by RNase and Actinomycin D in comparatively high concentrations. Soluble DNA polymerase was extracted from buffered suspensions of the nuclei by centrifugation at 100,000 *g*. It possessed properties very similar to those exhibited by the enzyme in intact nuclei. The polymerase activity of intact nuclei was increased by freezing and thawing, and the soluble enzyme was unstable at 30 C and lost most of its activity in 1 day at 0 C. The specific activity of the enzyme was 10–29 picomoles of deoxynucleoside triphosphate incorporated into DNA by 1 mg of protein in 1 hr at 25 C.

Received October 6, 1969

INTRODUCTION

SINCE THE DISCOVERY of deoxyribonucleic acid (DNA)[2] polymerase (deoxynucleoside triphosphate:DNA deoxynucleotidyl transferase, EC 2.7.7.7.) in Kornberg's laboratory (Lehman et al., 1958), the enzyme has been purified, sometimes extensively, from bacterial cells (Richardson et al., 1964; Litman, 1968) and animal tissues (Kier, 1965; Yoneda and Bollum, 1965). Much of the work on mammalian DNA polymerase has been carried out with rat liver. Thus, soluble DNA polymerase has been prepared from whole, usually regenerating, rat liver tissue (Bollum, 1958; Mantsavinos, 1964), and also from rat liver nuclei (Mantsavinos and Canellakis, 1959). Recently, Kalf and Ch'ih (1968) have shown that rat liver nuclei possess a DNA polymerase that appears to be distinct from that present in mitochondria isolated from the same tissue. The present communication outlines some of the properties of the DNA polymerase of trout (*Salmo gairdneri*) liver nuclei.

MATERIALS AND METHODS

The trilithium salts of the nonradioactive deoxynucleoside triphosphates used were prepared as described by Smith and Khorana (1963). Tritiated thymidine triphosphate (TTP^3H) was pur-

[1]The results formed part of a paper presented at the Annual Meeting of the Canadian Biochemical Society, Edmonton, Alta., June 11–13, 1969.

[2]The following abbreviations are used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; dATP, deoxyadenosine triphosphate; dGTP, deoxyguanosine triphosphate; dCTP, deoxycytidine triphosphate; TTP, thymidine triphosphate; DNase, deoxyribonuclease; RNase, ribonuclease; cpm, counts per minute.

Printed in Canada (J1610)

chased from Schwarz Laboratories. The purity of these compounds was checked before use by paper chromatography. Bovine pancreatic ribonuclease (5× crystallized), deoxyribonuclease (type IA, once crystallized), yeast RNA (type VI), and calf thymus sodium DNA (type V) were obtained from Sigma. Actinomycin D and dithiothreitol were purchased from Calbiochem.

DNA polymerase activity was determined by measuring the incorporation of TTP^3H into DNA under the following conditions. The reaction mixture contained, in a final volume of 0.5 ml, 100 nmoles each of nonradioactive dATP, dGTP, and dCTP; 2.31 nmoles of TTP^3H (1.25 μ Ci); 50 μmoles tris (hydroxymethyl) amino methane–HCl buffer, *p*H 7.7; 50 μg thymus DNA primer (heated 10 min at 100 C in 10 mM tris *p*H 7.5 and promptly cooled); 1 μmole dithiothreitol and 2 μmoles $MgCl_2$. Suspensions of intact trout liver nuclei (Tarr and Gardner, 1970) or of soluble DNA polymerase prepared from them (see below) were added in 0.1–0.3 ml amounts in different experiments. The amounts of nuclei or of soluble enzyme preparation employed in experiments are recorded as milligrams of DNA and milligrams of protein, respectively. The reaction mixtures were held 1 hr at 25 C unless otherwise stated. The solutions were then chilled and the radioactivity of the acid soluble DNA-containing fraction was determined by the method Widnell and Tata (1966) employed in their study of RNA polymerase. For convenience, RNA was used as carrier in all but a few experiments where the incorporation of radioactivity into the thymine moiety of DNA was investigated and, in these, Sigma DNA was employed. The radioactivities of the DNA-containing solutions were determined as described elsewhere (Tarr and Gardner, 1970). Protein was determined by the quantitative biuret reaction (Kingsley, 1939) and DNA by the method of Burton (1956).

RESULTS AND DISCUSSION

Experiments with Intact Nuclei

Figure 1 shows that the DNA polymerase activity of intact trout liver nuclei was about the same between *p*H 6.5 and 8.0, and that it decreased at higher *p*H values. Incorporation of TTP^3H into DNA by intact nuclei proceeded at an approximately constant rate for 3 hr at 25 C (Fig. 2).

Table 1 shows the effect of certain inhibitors and of freezing and thawing on the DNA polymerase of intact liver nuclei. The withdrawal of one or more deoxynucleoside triphosphates from the assay medium occasioned marked inhibition of DNA synthesis. Elimination of $MgCl_2$ or of primer DNA also

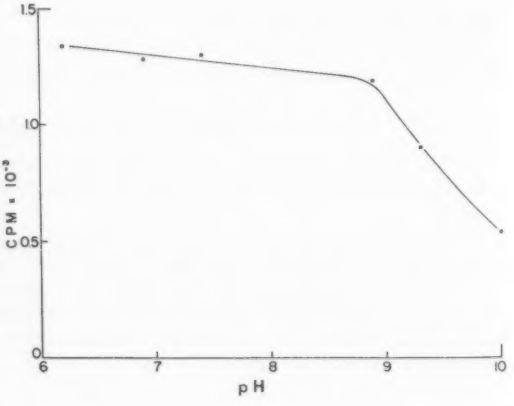

FIG. 1. *p*H-activity relationship of DNA polymerase in intact trout liver nuclei. The conditions used were those of the standard assay with Tris–HCl buffers in 0.1 mM concentration; 0.41 mg nuclear DNA per experiment. The *p*H of identically prepared solutions was determined at 25 C immediately before conducting experiments.

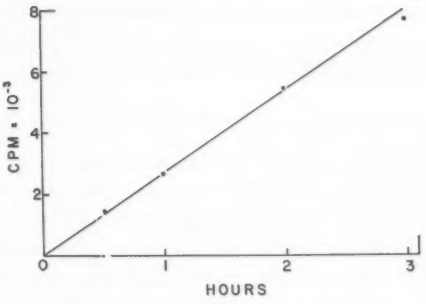

FIG. 2. Effect of time on DNA biosynthesis by intact trout liver nuclei; 0.5 mg nuclear DNA per experiment.

TABLE 1. The various inhibitors were added to tubes containing the components of the standard assay at 0 C before incubating them 1 hr at 25 C.

	Increase or decrease in incorporation of TTP^3H into DNA (%)	Mg nuclear DNA/expt
Complete assay medium	None	
Minus dGTP	−38	0.23
Minus dGTP	−41	0.18
Minus dGTP, dCTP, and dATP	−82	0.23
Minus dGTP, dCTP, and dATP	−82	0.18
1 μg DNase	None	0.21
2 μg DNase	−22	0.21
5 μg DNase	−85	0.18
10 μg DNase	−90	0.18
10 μg RNase	None	0.21
50 μg RNase	−33	0.21
10 μg Actinomycin D	−37	0.18
10 μg Actinomycin D	−39	0.21
40 μg Actinomycin D	−49	0.18
50 μg Actinomycin D	−63	0.21
2 μmoles $Na_4P_2O_7$	−100	0.21
5 μmoles $Na_4P_2O_7$	−100	0.21
Minus $MgCl_2$	−49	0.21
Frozen and thawed once	+150	0.23
Frozen and thawed three times	+164	0.23

strongly retarded polymerase activity. Comparatively low concentrations of DNase were strongly inhibitory, and pyrophosphate caused complete inhibition. Actinomycin D and RNase were inhibitory only in comparatively high concentrations. The inhibitory effect of RNase is apparently not due to its contamination with DNase, since sensitive determinations, using ^{14}C-labeled

DNA prepared from *E. coli*, showed that DNA is not appreciably degraded by the RNase under the conditions of the standard assay. The inhibitory effect was also observed with soluble DNA polymerase (see below). Freezing and thawing caused a marked increase in activity.

Incorporation of TTP^3H into the Thymine of DNA in Intact Nuclei

Four of the DNA-containing extracts in which there had been good incorporation of TTP^3H into DNA were selected from the above experiments. In these, DNA and not RNA had been used as carrier during the isolation of the DNA-containing fractions. The fractions were pooled, adjusted to about *p*H 6.0, and the crude DNA was precipitated by addition of 2 vol of ethanol. It was dried in vacuum and hydrolyzed 1 hr at 100 C with 70% perchloric acid. The four constituent bases were separated by paper chromatography, eluted, dissolved in water, identified by spectrophotometric determinations, and their radioactivities determined as previously described (Tarr and Roy, 1966). Only the thymine was radioactive (specific activity, 4000 counts per min per μmole) showing that the TTP^3H had been specifically incorporated into the thymine moiety of the DNA.

Soluble DNA Polymerase from Trout Liver Nuclei

Several different treatments of suspensions of the nuclei, which it was hoped would facilitate liberation of soluble DNA polymerase, were investigated. These included repeated freezing and thawing, prolonged homogenization with a "Tri R" homogenizer, exposure to vacuum, and treatment in a Bronwill homogenizer using 4-mm glass beads. The treated suspensions were subsequently centrifuged. The clear supernatant solutions were no more active than those obtained by centrifuging the suspensions of nuclei at 100,000 *g* for 30 or 60 min without prior treatment. This is demonstrated by the following experiment.

Two similar portions of trout liver nuclei were obtained from 5 g of liver. One portion was suspended in 2 ml of 10 mM Tris–HCl buffer (*p*H 7.7) containing 1 mM $MgCl_2$ and 1 mM dithiothreitol, and the suspension was exposed to fairly high vacuum for 3 min. The second portion was suspended in 250 mM sucrose containing $MgCl_2$ and dithiothreitol as above. Both suspensions were centrifuged 30 min at 100,000 *g*. The clear supernatant solutions were decanted and the residues resuspended in 2-ml portions of identical solutions and centrifuged again as before. Finally, the residues were suspended again in the buffered solutions. The solutions and suspensions were tested for DNA polymerase activity using 0.3-ml portions in the standard assay procedure.

The results (Table 2) show that, in both extraction procedures, much of the DNA polymerase activity was obtained in the initial extracts, the second extracts possessing consistently lower activities. The residues had only about 20% of the total activity. Ultracentrifugation of the nuclear suspension without prior treatment gave preparations with activities about the same as those from the pretreated nuclei.

TABLE 2. Comparative DNA polymerase activities of fractions of trout liver nuclei.

	Nuclei suspended in Tris buffer, vacuum treated, and ultracentrifuged			Nuclei suspended in sucrose buffer and ultracentrifuged		
	Protein (*mg/ml*)	% of total protein in extract	S.A.[a] of extract	Protein (*mg/ml*)	% of total protein in extract	S.A. of extract
First extraction	1.50	17.1	5600	1.28	14.8	6600
Second extraction	0.80	9.1	2700	1.14	13.2	2100
Suspension of residue	6.50	74.8	1800	6.25	72.0	1600

[a]S.A., specific activity in cpm per milligram protein per hour at 25 C.

EFFECT OF TEMPERATURE AND $MgCl_2$ ON THE SOLUBLE ENZYME

Figure 3 shows that, at 25 C, the enzyme exhibited constant activity for 4 hr. At 30 C, the rate of DNA synthesis was about the same as at 25 C for the first 2 hr, but thereafter slowly decreased, presumably due to enzyme inactivation.

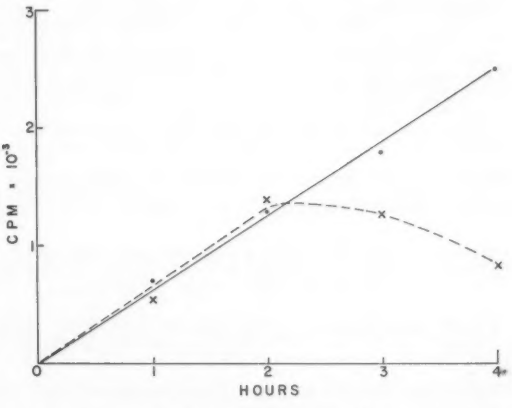

FIG. 3. Effect of time and temperature on DNA biosynthesis by soluble DNA polymerase of trout liver nuclei; 0.29 mg protein per experiment; •—•, 25 C; ×---×, 30 C.

Experiments showed that the enzyme had a marked Mg^{2+} requirement (optimum concentration 2–10 mM). Withdrawal of $MgCl_2$ from the assay medium, which resulted in a final concentration of 0.4 mM $MgCl_2$ due to the $MgCl_2$ used in the preparation of nuclear suspension, caused an inhibition of about 75% (Table 3).

INHIBITION OF SOLUBLE DNA POLYMERASE

The effect of inhibitors used in experiments with soluble enzyme are recorded in Table 3. In general, the results were closely similar to those obtained with intact nuclei. They demonstrated marked sensitivity to withdrawal of one or more deoxynucleoside triphosphates, and strong inhibition by DNase and pyrophosphate. Comparatively high concentrations of Actinomycin D and

TABLE 3. Inhibition of soluble DNA polymerase from trout liver nuclei

	Decrease in incorporation of TTP^3H into DNA (%)	Mg protein/expt
Complete assay medium	None	0.29 and 0.64
Minus dATP	−57	0.29
Minus dGTP	−61	0.64
Minus dATP and dCTP	−69	0.29
Minus dATP, dCTP, and dGTP	−95	0.29
Minus dATP, dCTP, and dGTP	−100	0.64
5 μg Actinomycin D	−81	0.29
10 μg Actinomycin D	−85	0.29
40 μg Actinomycin D	−100	0.64
0.5 μmole $Na_4P_2O_7$	−85	0.29
1.0 μmole $Na_4P_2O_7$	−89	0.29
3.0 μmole $Na_4P_2O_7$	−100	0.29
1 μg DNase	−56	0.29
2 μg DNase	−68	0.29
5 μg DNase	−98	0.29
20 μg RNase	−30	0.64
20 μg RNase	−80	0.29
40 μg RNase	−83	0.29
40 μg RNase	−64	0.64
24 hr at 0 C	−74	0.64
24 hr at 0 C	−98	0.29
Minus $MgCl_2$	−75	0.57
Minus DNA	−98	0.57

RNase were required to inhibit the polymerase activity. Elimination of DNA caused almost complete inhibition.

COMPARATIVE ACTIVITIES OF TROUT LIVER NUCLEI DNA POLYMERASE AND OF SIMILAR ENZYMES FROM MAMMALIAN TISSUES

A survey of six different preparations used in the present work showed that 10–29 picomoles of TTP^3H (or $dATP^3H$) were incorporated into the acid insoluble fraction by the soluble extract of trout liver nuclei per milligram protein per hour at 25 C. For mammalian systems, the following figures (picomoles per milligram protein per hour at 37 C) have been reported: (1) a 10-fold purified enzyme from regenerating rat liver, 430 (Bollum, 1958); intact rat liver mitochondria, 2.0, and a 24-fold purified enzyme from this source, 1350 (Kalf and Ch'ih, 1968); crude extract of rat liver, 2.0, and a partially purified fraction from this source, 1900 (Mantsavinos, 1964); crude extract of calf thymus, 1600, and about 150-fold purified enzyme, 250,000 (Yoneda and Bollum, 1965). Thus, for crude liver preparations, the incorporation is in general quite low, as it is for the present preparation from fish liver nuclei. Work in progress in this laboratory has resulted in partial purification of a soluble DNA polymerase from immature sockeye salmon testes, which has a specific activity of 25,000–45,000.

REFERENCES

BOLLUM, F. J. 1958. Incorporation of thymidine triphosphate into deoxyribonucleic acid. J. Amer. Chem. Soc. 80: 1766–1777.

BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62: 315–323.

KALF, G. F., AND J. J. Ch'ih. 1968. Purification and properties of deoxyribonucleic acid polymerase from rat liver mitochondria. J. Biol. Chem. 243: 4904–4916.

KIER, H. M. 1965. DNA polymerases from mammalian cells, p. 81–128. *In* J. N. Davidson and W. E. Cohn [ed.] Progress in nucleic acid research and molecular biology, Vol. 4. Academic Press, Inc., New York.

KINGSLEY, G. R. 1939. The determination of serum total protein, albumin, and globulin by the biuret reaction. J. Biol. Chem. 131: 197–200.

LEHMAN, I. R., M. J. BESSMAN, E. S. SIMMS, AND A. KORNBERG. 1958. Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *Escherichia coli*. J. Biol. Chem. 233: 163–170.

LITMAN, R. M. 1968. A deoxyribonucleic acid polymerase from *Micrococcus leuteus* (*Micrococcus lysodeikticus*) isolated from deoxyribonucleic acid-cellulose. J. Biol. Chem. 243: 6222–6233.

MANTSAVINOS, R. 1964. Studies in the synthesis of deoxyribonucleic acid by mammalian enzymes. I. Incorporation of deoxyribonucleoside 5'-triphosphates into deoxyribonucleic acid by a partially purified enzyme from regenerating rat liver. J. Biol. Chem. 239: 3431–3435.

MANTSAVINOS, R., AND E. S. CANELLAKIS. 1959. Studies on the biosynthesis of deoxyribonucleic acid by soluble mammalian enzymes. J. Biol. Chem. 234: 628–635.

RICHARDSON, C. C., C. L. SCHILDERKRAUT, H. V. APOSHIAN, AND A. KORNBERG. 1964. Enzymatic synthesis of deoxyribonucleic acid. XIV. Further purification and properties of deoxyribonucleic acid polymerase of *Escherichia coli*. J. Biol. Chem. 239: 222–231.

SMITH, M., AND H. G. KHORANA. 1963. Preparation of nucleotides and derivatives, p. 645–669. *In* S. P. Colowick and N. O. Kaplan [ed.] Methods in enzymology, Vol. 6. Academic Press, Inc., New York.

TARR, H. L. A. ,AND L. GARDNER. 1970. Ribonucleic acid synthesis by salmon liver nuclei. Int. J. Biochem. (In press.)

TARR, H. L. A., AND J. ROY. 1966. Incorporation of radioactive compounds into the purine and pyrimidine bases and 2-deoxyribose of salmon testes deoxyribonucleic acid. Can. J. Biochem. 44: 1435–1446.

WIDNELL, C. C., AND J. R. TATA. 1966. Studies in the stimulation by ammonium sulphate of the DNA-dependent RNA polymerase of isolated rat liver nuclei. Biochim. Biophys. Acta 123: 478–492.

YONEDA, M., AND F. J. BOLLUM. 1965. Deoxynucleotide-polymerizing enzymes of calf thymus gland. I. Large scale purification of terminal and replicative deoxynucleotidyl transferases. J. Biol. Chem. 240: 3385–3391.

Long-Term Effects of Diet on the Biology of the Lake Trout and the Fishery in Lake Opeongo, Ontario[1]

N. V. MARTIN

Ontario Department of Lands and Forests
Research Branch, Maple, Ont.

MARTIN, N. V. 1970. Long-term effects of diet on the biology of the lake trout and the fishery in Lake Opeongo, Ontario. J. Fish. Res. Bd. Canada 27: 125–146.

From 1936 to 1965 food content was examined in 17,171 Lake Opeongo lake trout (*Salvelinus namaycush*). The diet is analysed from the point of view of frequency of occurrence of foods, their weight, feeding activity of trout, size of trout and size of food, and seasonal and annual changes in food habits.

In the late 1940's and early 1950's perch, *Perca flavescens*, largely disappeared from the diet. The lake trout initially turned to lake whitefish, *Coregonus clupeaformis*, and then to the cisco, *Coregonus artedii*, introduced into Lake Opeongo in 1948.

As a result of these dietary changes lake trout are now heavier and rate of growth has improved. Age and size at maturity is later and there are fewer infertile fish. Egg counts, egg size, and ovary weights are now greater, particularly in the medium-sized lake trout.

In the fishery the average weight of individual trout taken has almost doubled. About 25% more of the immature stock is exploited by the recent fishery. The harvest and catchability of lake trout has remained generally constant on a numerical basis but show an improvement on a poundage basis in the more recent years. The possible effects of these changes on the character of the fishery are discussed.

Received August 12, 1969.

INTRODUCTION

LAKE OPEONGO, Algonquin Park, Ont., first became readily accessible to anglers in 1936 and since that date a creel census has been carried out for lake trout, *Salvelinus namaycush*. The total reported annual catch has varied between 500 and 2000 with a mean figure of about 1000 trout per year for the period 1936–65.

In addition to the collection of catch statistics, approximately 60% of the lake trout have been examined for food content and data have also been obtained on weights, ages, maturity, and gonad development. Gillnetting, trapnetting, and ice fishing have provided additional material for the present analysis.

This report provides a long-term study of lake trout food habits in one lake, illustrating the importance of diet on the general biology of the species, and its indirect effects on the fishery itself.

---

[1]Contribution No. 69-7 of the Ontario Department of Lands and Forests, Research Branch, Maple, Ont.

Printed in Canada (J1565)

FOOD OF THE LAKE TROUT

Frequency of Occurrence of food

In the period 1936–65 a total of 17,171 lake trout were examined for food content. Although every month but December is represented in the samples about 90% of the material was collected in the May to September period inclusive. In Table 1 the organisms or groups of organisms found in the stomachs have been listed with their percentage frequency of occurrence. As will be shown changes have occurred in the diet of Opeongo lake trout and the time spans in the table represent the major shifts that have taken place in the food habits over the 36 years of the study. Fish have been the most important element, being found in about 60% of the lake trout stomachs. Yellow perch (*Perca flavescens*), lake whitefish (*Coregonus clupeaformis*), and cisco or lake herring (*Coregonus artedii*) have been the most important fish foods although the relative importance of each species has changed substantially over 30 years. Various cyprinids, burbot (*Lota lota*), smallmouth bass (*Micropterus dolomieui*), round whitefish (*Prosopium cylindraceum*), brook trout (*Salvelinus fontinalis*), pumpkinseed (*Lepomis gibbosus*), American eel (*Anguilla rostrata*), brown bullhead (*Ictalurus nebulosus*), and hybrid trout (*Salvelinus namaycush* × *S. fontinalis*) are also occasionally eaten by the lake trout.

Insects are taken by lake trout a fifth as frequently as fish. Aquatic insect forms are eaten three times as often as are terrestrial. Ephemeroptera, particularly the burrowing mayflies, are the dominant insect food in Opeongo lake trout. Various Diptera, most of which are taken planktonically in pupal form, are also significant in the insect diet. In addition to these orders itemized in the table, various representatives of the Plecoptera, Coleoptera, and Hemiptera are rarely taken. The Hymenoptera, and in particular the flying ants (Formicidae), are the main terrestrial insect food. Coleoptera, chiefly the June bugs (Scarabaeidae), are also frequently eaten by the lake trout. Representatives of the Trichoptera, Orthoptera, Lepidoptera, Odonata, Ephemeroptera, Homoptera, and Heteroptera were infrequently taken at the surface by the lake trout.

The freshwater sponge, *Spongilla*, is commonly found in lake trout stomachs. Plankton in the table refers chiefly to the planktonic Crustacea, largely cladocerans and copepods. Dipterous pupae and *Chaoborus* larvae (included in the insect food) may also be taken planktonically. Other miscellaneous items periodically observed in the diet were frogs, salamanders, spiders, molluscs, shrews, earthworms, and fish eggs.

Amount of Food

A quantitative measure of food consumed by Opeongo lake trout is presented in Table 2. These data can only be regarded as estimates because of widely differing rates at which the various food items are digested. Perch, although occurring as frequently as whitefish, contribute only one-quarter

TABLE 1. Number and percentage frequency of occurrence of food items and empty stomachs in Opeongo lake trout for the years 1936–50, 1951–56, 1957–65, and 1936–65.

Food item	1936–50 Total no. and %	1951–56 Total no. and %	1957–65 Total no. and %	1936–65 Total no. and %
A. Major groups of food eaten				
Total no. lake trout examined	9,127	3,267	4,777	17,171
Trout with fish	62.3	56.8	56.1	59.6
" " insects	18.5	8.3	3.2	12.3
Aquatic stages	78.7[a]	76.7[a]	70.2[a]	77.8[a]
Terrestrial stages	27.0[b]	14.8[b]	4.1[b]	23.9[b]
" " freshwater sponge	1.4	0.4	0.0	0.8
" " plankton	0.9	0.4	3.3	0.6
" " detritus[c]	1.0	0.2	0.0	0.6
" " crayfish	0.4	0.3	0.1	0.3
" " leeches	0.1	0.2	0.1	0.1
" " other items	0.2	<0.1	<0.1	0.1
" " empty stomachs	27.2	36.9	36.3	31.6
B. Species of fish and orders of insects represented in food				
Trout with fish in stomach (totals)	5,686	1,857	2,683	10,226
Yellow perch (*Perca flavescens*)	41.9	11.1	5.1	26.6
Lake whitefish (*Coregonus clupeaformis*)	33.3	26.2	7.0	25.1
Cisco (*Coregonus artedii*)	0.2	2.6	25.0	7.2
Unidentified coregonids	0.6	1.5	11.6	3.6
Slimy sculpin (*Cottus cognatus*)	2.3	1.5	1.0	1.8
White sucker (*Catostomus commersoni*)	2.2	0.6	0.2	1.4
Other (each less than 1%)	2.6	2.0	0.9	2.0
Unidentified fish remains	34.6	65.6	62.6	47.6
Trout with aquatic insects in stomach (totals)	1,330	207	106	1,643
Ephemeroptera	65.3	84.1	53.8	67.0
Diptera	34.4	20.8	5.7	30.8
Odonata	6.2	0.5	2.8	5.3
Neuroptera	3.5	0.0	0.0	2.8
Trichoptera	2.9	0.0	2.8	2.5
Other (each less than 1%)	0.9	0.0	1.9	0.9
Unidentified aquatic insects	0.9	1.5	31.1	2.9
Trout with terrestrial insects in stomach (totals)	457	40	7	504
Hymenoptera	55.1	55.0	28.6	54.8
Coleoptera	31.3	5.0	0.0	28.8
Diptera	9.4	5.0	0.0	8.9
Hemiptera	2.2	2.5	0.0	2.2
Homoptera	1.3	0.0	0.0	1.2
Other (each less than 1%)	2.8	0.0	14.2	2.8
Unidentified terrestrial insects	12.0	32.5	14.2	13.7

[a]Of the trout with insects in stomach the percentage that had ingested aquatic insects.
[b]Of the trout with insects in stomach the percentage that had ingested terrestrial insects.
[c]Detritus included vegetation, bark, stones, etc.

TABLE 2. Average number, size, and weight of some major food organisms per lake trout stomach, Lake Opeongo, 1936–65.

Item	Avg no.	Avg size (*inches*)	Avg wt (g)
Total fish			18.2
Whitefish	1.6	7.5	29.2
Perch	3.8	1.8	7.3
Cisco	1.4	5.2	21.5
White sucker	1.1	9.7	98.6
Burbot	1.2	7.7	62.2
Slimy sculpin	1.7	1.8	2.8
Unidentified coregonids	1.4	5.6	25.4
Unidentified remains			9.8
Other species			40.2
Total insects			3.1
Total aquatic insects			2.5
Mayfly nymphs	4.5		1.7
Dipterous pupae	4.0		0.3
Dragonfly nymphs	1.1		0.7
Unidentified aquatic			0.4
Other aquatic			0.7
Total terrestrial insects			7.1
Hymenoptera	Numerous		9.6
Unidentified Coleoptera			3.0
Scarabaeidae	3.3		3.1
Unidentified terrestrial			6.0
Other terrestrial			2.4
Freshwater sponge			1.4
Crayfish	1.5		2.9
Plankton			0.8

the bulk by weight. White sucker and burbot are infrequently eaten but because of their size provide considerable weight of food to the individual trout.

Terrestrial insects are found in the stomachs only a third as often as aquatic insects but contribute three times the weight to the individual trout. Engorgement of the lake trout on periodic large hatches of flying ants, June bugs, and March flies (Bibionidae) account for this difference.

SEASONAL FOOD HABITS

Figure 1 shows the percentage frequency of occurrence of various organisms, groups of organisms, and empty stomachs for each half-month of the year in the period 1936–65. Although variations occur in different years, a general pattern is evident that follows that described by Martin (1952, 1954) in other Algonquin Park lakes.

During the winter about half of the lake trout have food in their stomachs consisting largely of fish. The most active feeding period of the year occurs in the spring (late April and May) just after the breakup of the ice. Insects, particularly mayfly nymphs and flying ants, are at a peak in abundance as

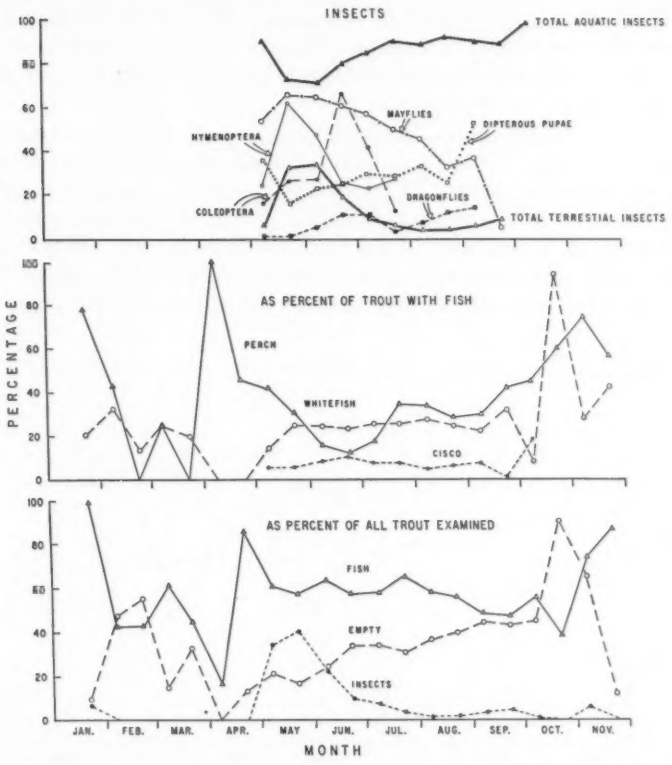

FIG. 1. Percentage frequency of occurrence of foods and empty stomachs in Lake Opeongo lake trout by half-months for the period 1936–65. In upper panel total aquatic insects and total terrestrial insects are shown as percentages of all trout with insects in stomachs. Specific insect forms shown as percentages of total aquatic or total terrestrial insects.

is fish content. As would be expected crayfish, minnows, and other shallow water forms are also most common at this time.

As the lake trout begin their migration to deeper waters in June, there is a sharp drop in the presence of insects in the stomachs. Beetles and dragonfly nymphs, however, reach peaks in this period. Fish content remains fairly constant through the early summer whereas the incidence of empty stomachs gradually increases. Perch are at a low level in late spring in the trout stomachs but then are found more often as perch-of-the-year enter the diet in mid-July. Whitefish and cisco occur at a constant rate throughout the spring and summer months. The figures for cisco appear low as percentages were computed for the whole 30 years rather than for the 10 years since its establishment in the lake. The curve, however, still indicates its relative seasonal abundance in the trout stomachs. Dipterous pupae, taken planktonically, are an important

part of the insect fauna consumed in summer. A peak in the percentage of nonfeeding fish and corresponding low points in fish, insect, and miscellaneous foods occurs in early fall. Small secondary peaks are evident in some elements of the insect fauna at this time.

In Fig. 2 a comparison is made of the feeding activity of mature and immature lake trout. This comparison is based on trout 16–19.9 inches in order to minimize any major bias because of the differences in the food habits of small and large fish. A similar comparison with the reduced size range of 16–17.9 inches for a number of years gave the same results. From early June until late August fish that will spawn that fall feed more consistently than those that will not. By early October mature fish almost entirely cease feeding

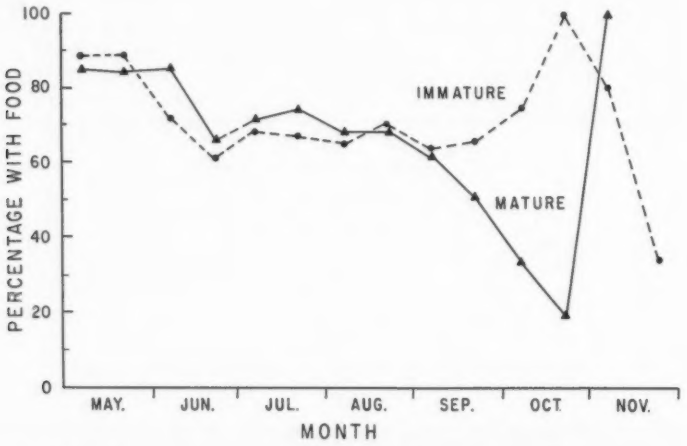

FIG. 2. Percentage frequency of occurrence by half-months of immature and mature lake trout in Lake Opeongo with food in stomachs. Based on trout 16–19.9 inches (fork length).

whereas nonspawning lake trout feed more actively. After spawning, which normally occurs in the last half of October, both groups are feeding at about the same intensity. Only eight immature fish were examined in late November and the low percentage of feeding trout at this time may be a result of the small sample.

ANNUAL CHANGES IN FOOD HABITS

Figure 3 shows the percentage frequencies with which fish, insects, and empty stomachs appear over the 30-year span of the study. The comparison for fish and empty stomachs is made for July and August only because sample sizes vary considerably as do the food habits for other months. In addition these months are critical with respect to food availability. As the consumption

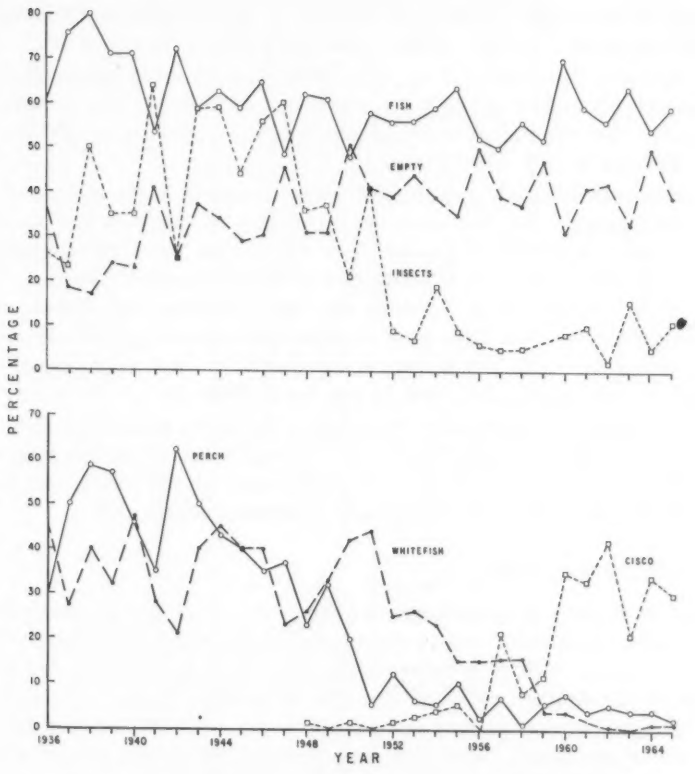

FIG. 3. Annual percentage frequency of occurrence of forage fish, insects, and empty stomachs in Lake Opeongo lake trout, 1936–65. Upper panel as percentage of all trout examined, lower panel as percentage of total lake trout with fish in stomachs.

of insects is largely confined to the last half of May and June the comparison is made for this period.

Although varying considerably from year to year the frequencies of occurrence of fish and empty stomachs have maintained the same general average, at least since the mid 1940's. Insect food, however, has shown a sharp decline since the early 1950's. The establishment of the cisco population about this time and its subsequent heavy utilization by the lake trout in the spring may account in part for this apparent decline in insect feeding.

Major yearly fluctuations in the frequencies of occurrence of empty stomachs, fish, and insects are evident. The percentage of empty stomachs has varied from 16 to 51% and as is apparent from the graph these values are generally inversely related to the percentage of stomachs containing fish food. In the earlier years this relationship was specifically associated with the perch. The presence of perch in general is in turn directly related to the

frequency of occurrence of perch-of-the-year in the stomachs. This age class has made up from 0 to 94% of the total perch diet over the years.

It has been demonstrated by Fry (1939) that a sharp thermocline may limit lake trout feeding on perch in the summer months. The present data also suggest that the size of entering year-classes of perch also affected trout feeding activity in the early years.

The most striking changes in food habits occurred in the decade of 1950. A sharp decrease in the frequency of occurrence of perch in the trout diet took place in the early 1950's. On the basis of quantitative studies (unpublished data) carried out in the years 1938–40 and 1959, this decrease reflects an actual decline in the abundance of perch in the lake. Whether this decline results from successive year-class failures or possibly competition with the introduced cisco is not known. With the decline of the perch the lake trout turned to the whitefish as their prime fish food in the years 1951–56.

In the spring of 1948 some 400 adult cisco were introduced into Lake Opeongo to provide additional forage for the lake trout. A few cisco appeared in the lake trout stomachs in the early 1950's and by 1960 they had become the dominant lake trout food and have continued so to date.

Size of Trout and Food

Table 3 shows the percentage frequency of occurrence of the more important food categories in various size groups of lake trout. The slightly higher incidence of empty stomachs in trout less than 15 inches may be due in part to the lower consumption of fish that would be retained longer in the stomachs than other food items. Sculpins and cyprinids are most often taken by trout in this size class. Small trout feed less often on terrestrial insects whereas plankton Crustacea is more commonly taken by this group.

In the medium-size groups of lake trout (15–24.9 inches) fish becomes more important in the diet, chiefly perch and whitefish. Insect feeding is at a higher level, particularly in the terrestrial forms. Mayfly nymphs are more commonly taken by this and the preceding size class whereas dragonfly nymphs do not occur as often as they do in the stomachs of the larger trout. The largest trout (over 25 inches) do not feed as often. As has been indicated earlier fish in this size range undergo a fasting period for about 2 months before spawning. Perch and whitefish are taken less often and cisco, white suckers, and burbot are found more frequently. Insect feeding is at a generally low level in the largest trout but when it occurs it is the terrestrial forms that are most commonly eaten.

Figure 4 shows the relationship between size of fish food and size of lake trout. For most fish species there is an increase in the size of the prey species with increase in predator size. In the perch, however, the young-of-the-year are extensively taken by all sizes of lake trout. These perch are generally first eaten at lengths of $\frac{3}{4}$–1 inch in early July up to lengths of 2 inches by the fall.

TABLE 3. Percentage frequency of occurrence of major food items and empty stomachs in various size classes (fork inches) of lake trout in Lake Opeongo, 1937–65.

Trout size class	No. examined	% with fish	As % of no. with fish[a]							% with Insects	As % of no.[a] trout with insects		As % of all trout examined			
			Perch	White-fish	Cisco	White sucker	Slimy sculpin	Burbot	Cyprin-ids		Aquatic	Terres-trial	Sponge	Plank-ton	Cray-fish	Empty
<15	2170	49.6	17.8	14.1	7.4	0.6	5.4	0.3	1.6	10.3	75.9	8.0	0.2	2.7	0.1	42.0
15–19.9	7908	61.9	31.4	24.1	4.6	0.5	1.7	0.2	1.0	15.6	75.1	24.5	1.0	0.6	0.2	30.1
20–24.9	4488	59.2	21.5	26.3	9.2	2.3	1.8	0.9	0.9	11.6	79.5	26.5	0.9	0.2	0.2	32.3
Over 25	1170	61.4	5.4	18.7	19.9	5.8	2.0	0.6	0.4	3.6	66.7	50.0	0	0.2	0.2	38.4

[a]As only some of the food items are shown and several items may occur in an individual trout stomach, total percentages may be less than or greater than 100%.

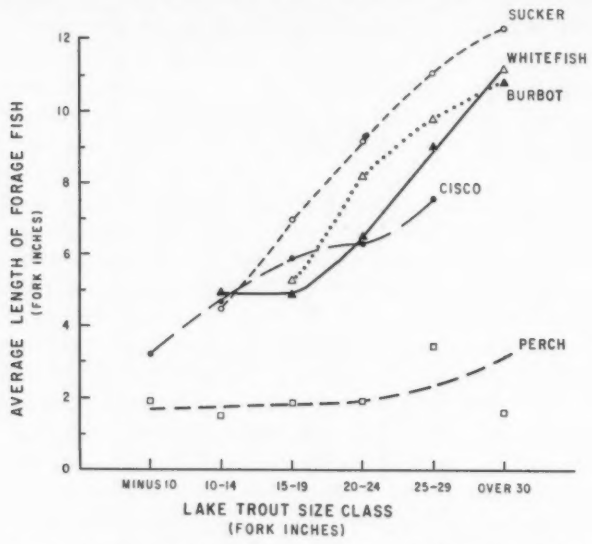

FIG. 4. Relationship between size of forage fish eaten and size of lake trout, Lake Opeongo, 1936–65.

As Table 4 indicates there is little relationship between the numbers of forage fish eaten and the size of lake trout. Lake trout tend to eat larger individuals of a species rather than more. Perch-of-the-year may be taken in large numbers by individual lake trout. For example a 21.5-inch, 60-oz lake trout contained 149 perch-of-the-year, an 18-inch, 40-oz trout 123, and a 17-inch, 25-oz trout 118. Perch-of-the-year by themselves averaged 6.0 individuals per lake trout stomach.

TABLE 4. Average number of forage fish species per lake trout stomach in various size groups of lake trout, Lake Opeongo, 1936–65. Values in parentheses indicate sample size of less than 15 lake trout.

Size group (*fork inches*)	Avg no. per lake trout stomach				
	Perch	Whitefish	Cisco	Common sucker	Burbot
<10	(18.0)	0	(1.0)	0	0
10–14.9	2.5	1.4	1.4	(1.0)	0
15–19.9	3.1	1.7	1.4	1.1	1.2
20–24.9	3.4	1.5	1.5	1.2	1.2
25–29.9	2.0	1.4	2.1	1.1	(1.2)
Over 30	(12.5)	(1.5)	(2.0)	1.0	(1.2)

Cannibalism

Cannibalism is apparently of little significance in Lake Opeongo. Only 11 lake trout were found in 17,171 lake trout stomachs examined (0.06%) and in three instances these were recently planted hatchery fish. Trout eaten by other lake trout were generally small although a 17-inch fish was found in the stomach of a 32-inch trout. A low incidence of cannibalism was noted by Martin (1952) in other Algonquin Park lakes and has been observed by other workers in a variety of localities.

EFFECTS OF FOOD CHANGES

The major transition in food habits took place in the late 1940's and early 1950's (see Table 1, Fig. 3) and the comparisons are therefore based on years before and after this period. Some minor variations occur in the specific dates selected for the comparisons because of the kind and amount of material available for analysis in different years.

Length–Weight, Age–Weight

In Fig. 5 the length–weight and age–weight relations of Lake Opeongo lake trout are shown for the periods 1937–47 and 1953–61 (1965).

Regression lines were fitted to the length–weight relation for the length range 12.5–25.5 inches (fork length). Sample sizes are more limited outside this range and inflections appear in the curves at these lengths. The equations of the lines are:

$$1937\text{–}47 \log W = -2.65266 + 3.37522 \log L$$
$$1953\text{–}65 \log W = -2.37848 + 3.09839 \log L$$

where W = body weight in ounces and L = fork length in inches. In this size range the trout in the 1953–65 period were significantly heavier for their length ($t = 4.16$, $P < 0.01$) than those in the earlier years, increments ranging up to 40%.

Because of the small sample sizes in the largest trout, statistical treatment of these data is not warranted. However, the extension of the 1953–65 regression line beyond the 25.5-inch length closely fits the points that have been plotted for these fish. The 1937–47 curve on the other hand shows a sharp inflection at this point. The length–weight relation of the large fish in both periods is similar, possibly because the food of these fish has remained basically the same (large whitefish, suckers, burbot) over both periods.

A slight inflection occurs in both logarithmic curves at about the 10-oz level. Small lake trout in the 1937–47 period are somewhat heavier for their length than those in later years. The virtual disappearance of the perch in the 1953–65 period, particularly the small perch-of-the-year on which these trout feed, may explain this difference.

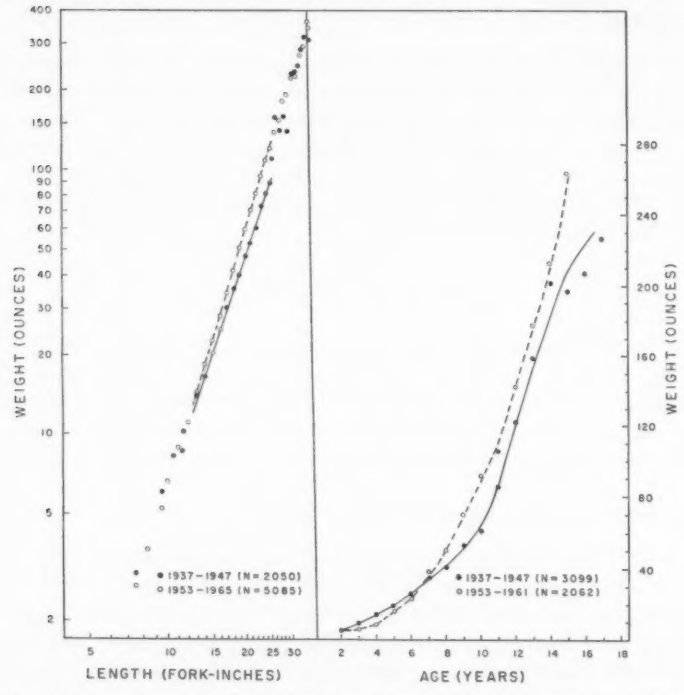

FIG. 5. Length–weight (logarithmic) and age–weight (arithmetic) relationships of Lake Opeongo lake trout for the years 1937–1947 and 1953–1961 (1965).

A similar pattern is evident in the arithmetic plot of the age–weight relationship although the curves do not coincide at the higher weights as they do in the length–weight relation. Lake trout of a given age vary greatly in their lengths and weights and the sample sizes are small for these old fish.

It is believed these changes in the length–weight and age–weight relations have resulted from the dietary changes that began in the late 1940's and early 1950's, that is, the decline of the perch population, the subsequent utilization of whitefish, and finally the dominance of cisco in the trout diet. This latter species is now extremely abundant in Lake Opeongo, readily available to the trout, and of forageable size. They are also probably superior to the perch and at least equal to the whitefish in nutritive value. A comparison of the length–weight data on a yearly basis indicates the trend towards increasing weight has levelled off over the last 4 years.

RATE OF GROWTH

Figure 6 shows rate of growth curves for Opeongo lake trout for the years 1937–49 and 1955–61. This age–length comparison parallels the situation in the length–weight and age–weight relations, that is, slower growth of the small

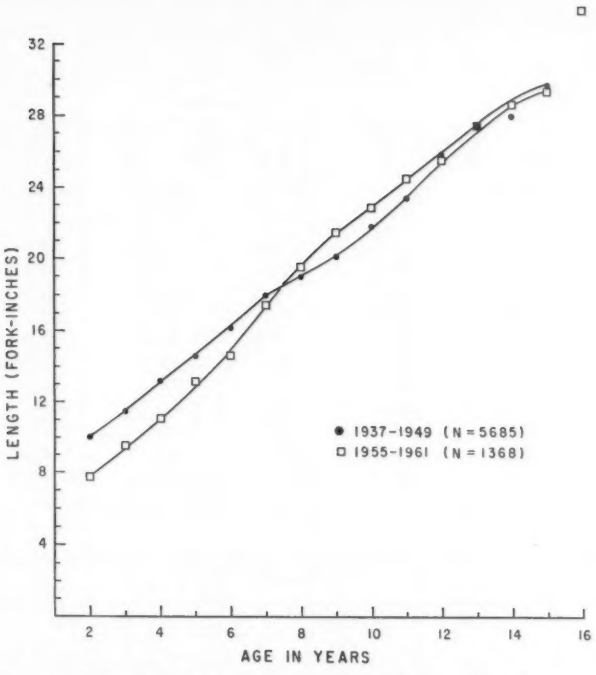

FIG. 6. Rate of growth of Lake Opeongo lake trout for the years 1937–49 and 1955–61.

fish in the 1955–61 period, faster growth of the medium-sized trout, and comparable growth in the oldest fish. In both weight and growth comparisons the depressions in the curves in the earlier years, particularly between ages 7 and 12, have been largely eliminated.

The effect of food changes on growth has been well documented for a number of fishes. In the lake trout McCaig and Mullan (1960) found increased growth rate in the lake trout in Quabbin Reservoir, Massachusetts, after the introduction and establishment of the smelt (*Osmerus mordax*). Bonneville cisco (*Prosopium gemmiferum*) have been introduced into Lake Tahoe (Frantz and Cordone, 1965) as forage for the lake trout and to improve growth rate but results are unknown to date. Similarly the opossum shrimp (*Mysis relicta*) was introduced into Lake Tahoe for the same reason (Hanson and Cordone, 1967).

MATURITY AND GONADS

Tables 5 and 6 present data on size and age at maturity of Lake Opeongo lake trout for the years 1937–48 and 1955–61 (1965). Only samples taken after August 15 have been used in the comparison so that the state of maturity of the gonads of this fall-spawning species could be more accurately determined.

TABLE 5. Percentage of lake trout mature at various lengths in Lake Opeongo, 1937–48 and 1955–65.

	% mature					
	Males		Females		Both sexes	
Length group (*fork inches*)	1937–48	1955–65	1937–48	1955–65	1937–48	1955–65
15–15.9	24.6	3.8	3.8	2.2	13.5	3.2
16–16.9	56.4	18.2	12.1	1.4	35.4	8.7
17–17.9	73.6	33.9	30.5	10.5	53.8	20.3
18–18.9	90.8	54.8	72.9	15.6	82.4	38.3
19–19.9	99.0	84.6	92.3	45.0	95.4	65.6
20–20.9	100	98.5	94.0	75.0	97.3	88.0
21–21.9	100	100	97.9	81.4	98.8	90.8
22–22.9	100	100	100	89.8	100	94.7
23–23.9	100	100	100	100	100	100
15–23.9	84.3	63.4	66.2	45.1	75.4	54.4
No.	1074	543	1027	515	2101	1058

TABLE 6. Percentage of lake trout mature at various ages in Lake Opeongo, 1937–48 and 1955–61.

	% mature					
	Males		Females		Both sexes	
Age (*years*)	1937–48	1955–61	1937–48	1955–61	1937–48	1955–61
4	0	0	0	0	0	0
5	16.7	4.0	11.1	0	13.9	2.2
6	50.5	25.0	17.6	0	33.5	12.3
7	85.6	60.5	56.3	18.6	71.6	39.5
8	95.9	77.5	86.9	65.9	91.6	71.6
9	100	96.3	98.1	81.3	99.0	88.1
10	100	100	100	100	100	100
No.	671	192	645	191	1316	383

There has been a general shift to later maturity in more recent years. In 1937–48, for example, over a third of the lake trout were mature at a length of 16–17 inches whereas in 1955–65 about 10% had reached maturity at this size. Similarly nearly three times as many trout attained maturity at age 6 in the earlier period as was the case in later years. No females have been found to be mature before age 7 in the 1955–61 period.

In Table 7 a comparison is made of the size and age composition of lake trout taken directly from the spawning beds in the years 1948–49 and 1961–62. These data corroborate those in Tables 5 and 6. Spawning lake trout in the

TABLE 7. Percentage length and age composition of lake trout taken from spawning beds in Lake Opeongo for the years 1948–49 and 1961–62.

Sex and years	Length group (*fork inches*)										Avg length	No.
	15–16.9	17–18.9	19–20.9	21–22.9	23–24.9	25–26.9	27–28.9	29–30.9	31–32.9	33–34.9		
Males												
1948–49	5.2	29.9	33.8	14.3	10.4	1.3	3.9	0	1.3	0	19.9	77
1961–62	0	0	19.8	18.8	24.0	13.5	14.6	6.2	0	3.1	23.9	96
Females												
1948–49	0	20.9	33.3	12.5	4.2	8.4	20.8	0	0	0	21.6	24
1961–62	0	0	2.3	24.0	36.8	20.3	11.3	3.8	1.6	0	24.1	133
Both sexes												
1948–49	3.9	24.0	37.6	15.2	8.0	3.0	7.2	0.7	0.7	0	20.4	138[a]
1961–62	0	0	9.6	21.8	31.4	17.4	12.6	4.9	4.9	1.3	24.0	229

	Age (*years*)										Avg age	No.
	6	7	8	9	10	11	12	13	14	15		
Males												
1948–49	6.4	12.8	29.8	25.5	14.9	8.5	0	2.1	0	0	8.7	47
1961–62	0	1.9	15.4	23.1	25.0	17.2	5.8	7.7	3.9	0	10.1	52
Females												
1948–49	0	6.3	6.3	50.0	6.3	6.3	12.5	12.5	0	0	9.9	16
1961–62	0	9.1	35.1	27.3	14.3	6.5	7.8	0	0	0	9.9	77
Both sexes												
1948–49	3.6	9.5	29.8	34.5	9.5	7.1	2.4	3.6	0	0	8.9	84[a]
1961–62	0	0.8	11.6	30.2	26.4	15.5	6.2	6.9	1.5	0.8	10.0	129

[a]Includes fish not sexed.

later years are 3–4 inches longer, and about a year older than those in the earlier period. Lake trout on the spawning beds averaged 6.85 lb in 1961 and 1962 whereas in 1948 and 1949 they averaged about half this weight.

Earlier maturity is generally associated with faster growth rate but the above data suggest the reverse. Similarly Martin (1966) found slow growing plankton-feeding trout matured earlier than fast growing trout in fish-feeding populations. Alm (1959) discussed the relation between maturity, size, and age in fish and concluded that although earlier maturity is usually related to faster growth rate, an initial good growth rate followed by a slower one may result in maturity at a smaller size and younger age. This latter situation prevailed in Lake Opeongo (Fig. 5 and 6) in the 1937–48 period, where the trout grew relatively fast initially and then exhibited a reduced growth rate. It is possible this growth pattern may explain the earlier maturity found in trout in Algonquin Park plankton-feeding populations and Lake Opeongo trout in the 1937–48 period.

Fry and Kennedy (1937) and Fry (1949) pointed out that Opeongo lake trout may fail to produce gametes in certain years after they have reached reached sexual maturity. These fish have been termed "infertile." Most are found in the 20–27 inch size range and the 9–13 age classes. As has been pointed out this infertility is not a permanent condition and the trout spawn again in later years.

In the present study a comparison was made of the percentages of infertile fish in the 20–30 inch size range in the years 1937–48 and 1955–65. Only fish collected after August 1 were used. In the earlier period 130 lake trout out of 784 examined (16.6%) were classified as infertile and in the latter period 16 out of 785 (2.0%). There has been a progressive decrease in infertility since 1955 and no infertile trout have been noted since 1960.

As has been previously observed there has been somewhat of a dietary lag in the food supply of the Opeongo lake trout and apparently this has also found expression in the general fertility of the fish. This situation has been apparently changed by the quality and quantity of forage now available to the lake trout.

In Fig. 7, 8, and 9 fecundity, egg size, and ovary weight data are presented for mature trout before and after the major food changes. Because of the variation in the length–weight relation, size at maturity, and rate of growth of the lake trout over the years, it is impossible to make a rigorous comparison for the two periods. It was felt, however, that fish of the same weight offered the best basis for comparison under these circumstances. A number of investigations have noted a higher degree of correlation between gonad development and fish weight than with length or age. Hanson and Wickwire (1967), for example, found this relationship for Lake Tahoe lake trout.

The average number of eggs per trout for the two periods is shown in Fig. 7. These counts were derived by the use of Fry's (1949) conversion diagram, which estimates egg numbers from egg diameter and ovary weight data. A similar procedure has been used by Vladykov (1956) for determining the

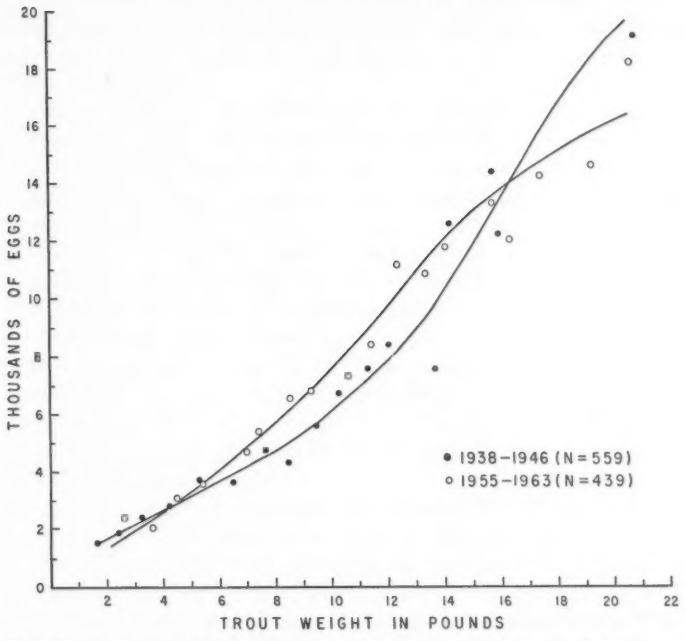

FIG. 7. Fecundity of Lake Opeongo lake trout for the years 1938–46 and 1955–63. Average number of eggs shown for lake trout at 1-lb intervals.

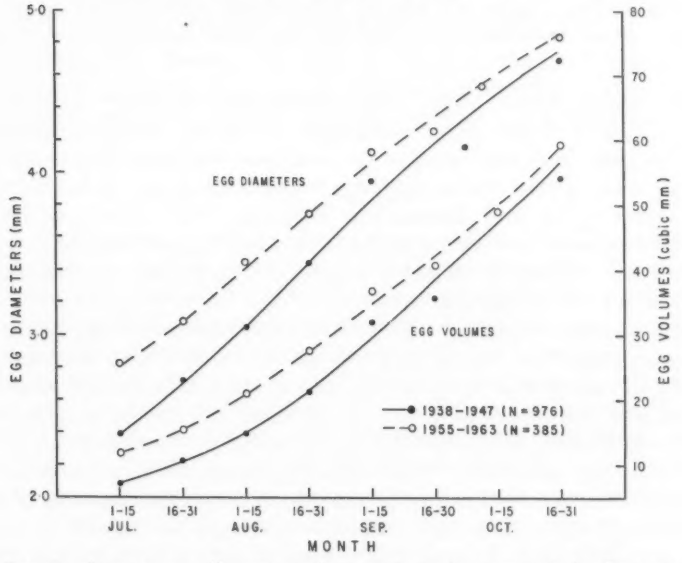

FIG. 8. Average egg diameter and egg volume in mature Lake Opeongo lake trout for the years 1938–46 and 1953–63 at 2-week intervals.

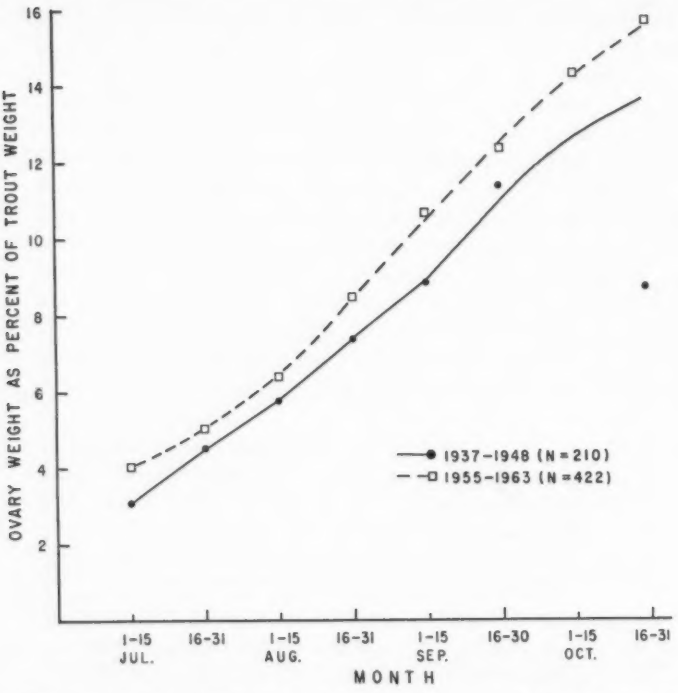

FIG. 9. Ovary weight of mature female trout as percent of total fish weight for years 1937–48 and 1955–63, by half-months.

fecundity of the eastern brook trout (*Salvelinus fontinalis*). As there is no clear indication of atresia in maturing eggs in Opeongo lake trout, egg counts from all months have been used in the analyses. Similarly Henderson (1963) found only small numbers of atretic eggs in the brook trout. As has been noted by Eschmeyer (1954) and Hanson and Wickwire (1967) lake trout fecundity is extremely variable between fish of the same age. The data from Lake Opeongo show this same variability. However, a generally curvilinear relation is evident despite the scatter of plotted values.

Egg production has been higher in the 1955–63 period for trout in the 4–16 lb size range but somewhat lower in the smaller fish. Because of the variability in counts and the small sample of large fish, considerable scatter in the points at the upper end of the curve is expected. As the relation is curvilinear it is difficult to state a specific figure for egg production on a per pound basis for Opeongo lake trout. In the 1938–48 period average fecundity figures ranged from 512 to 926 eggs per pound and in 1955–63 from 576 to 934. A general average for both periods would be 730 eggs per pound.

Figure 8 shows the diameter and volume of eggs of mature lake trout by 2-week periods. No consistent relation was found between egg size and fish

size and the trout of all weights have been grouped for the comparison. Similarly Eschmeyer (1954) found no such correlation between egg size and fish size in Lake Superior trout.

It is evident eggs are generally larger in the later years. Diameters have increased from 3 to 18% and volumes from 10 to 64%. The curves, however, indicate that final egg sizes for the two periods approximate each other.

In Fig. 9 ovary weight, relative to fish weight, is compared for the two time periods by half-months for trout from 3.3 to 19.2 lb. As was the case with egg numbers and egg size, ovary weights are quite variable. Some of the larger trout have proportionately heavier ovaries but there is no consistent trend. This same comparison was made for specific weight classes of lake trout and a similar pattern was evident.

Ovaries are generally larger in the later period. Gonad weights averaged from about 3% of the total fish weight in early July to about 14% in late October in the years 1937–48. In 1955–63 they averaged from 4.5 to over 15% at the same time of year. In general, female gonads were 0.5–2% heavier in the later years of the study.

These changes in gonad development are again felt to be related to changes in the food habits of the lake trout in Lake Opeongo. Numerous references attest to the effects of dietary changes on reproductive systems of fishes. Atwood (1960) has reviewed much of the literature in this field and has found that, in general, fecundity and egg size are greater under better feeding conditions. Vladykov (1956) found brook trout produced more eggs after the food supply in a lake became more abundant. Scott (1962), in his examination of experimental and natural populations of rainbow trout (*Salmo gairdneri*), found lower numbers of eggs in starved fish because of a higher degree of follicular atresia. Fry (1949) in his study of Opeongo lake trout suggested that "dietary conditions at the time the eggs are first laid down, probably two years previous to the one in which they mature, influence the number of eggs produced."

THE FISHERY

The panels in Fig. 10 show various aspects of the lake trout fishery in Lake Opeongo for the years 1936–65. Despite variations in the amount of fishing effort, the completeness of the census coverage, the skill of the anglers, and the strength of year-classes, certain trends are evident, some of which may be related to the dietary history of the trout in the lake.

Harvest figures (Fig. 10A) are quite variable and broadly related to the amount of fishing effort. The number of trout reported caught has fluctuated around a general mean of 1000 fish per year since the late 1940's although fishing effort (Fig. 10B) has shown an upward trend. The poundage taken, however, has increased over these years. There has been a consistent decrease in the numbers of trout caught per unit of fishing effort, whereas the pounds-per-hour index has remained relatively stable since the mid-1950's (Fig. 10C). The number of trout taken per acre has remained relatively consistent whereas

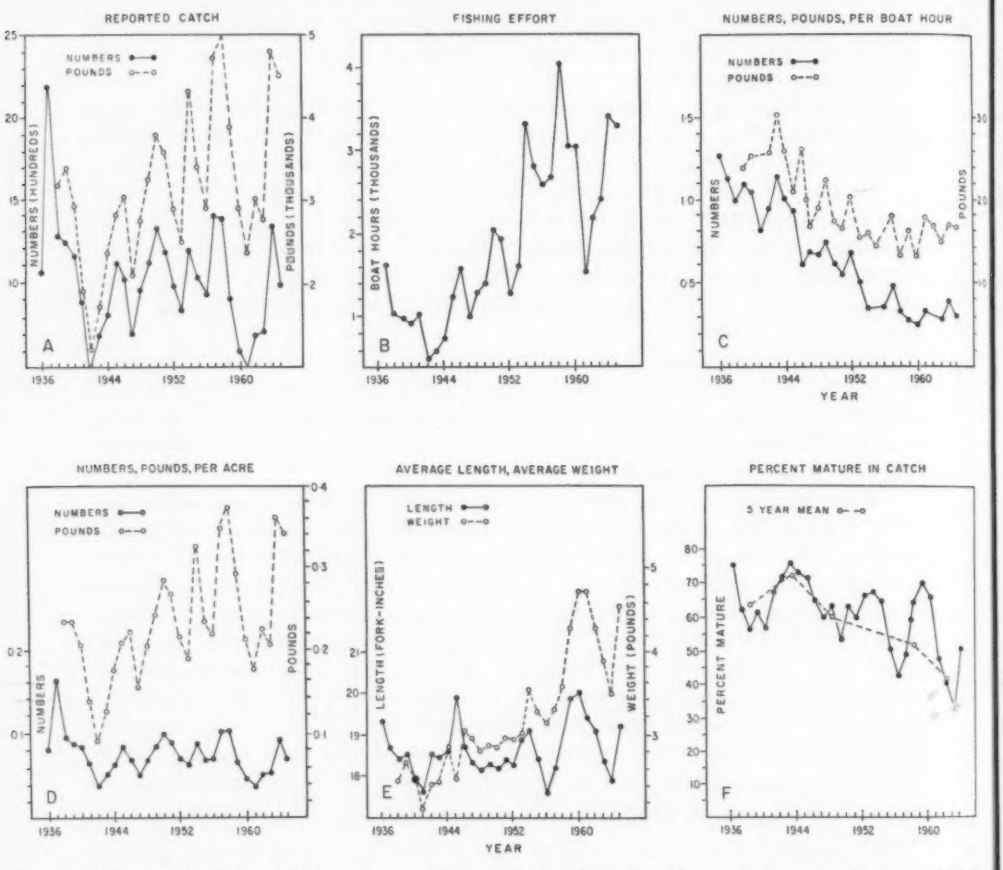

FIG. 10. Data on the Opeongo lake trout sport fishery obtained through creel census returns for the years 1936–65.

the poundage per acre has increased subtantially (Fig. 10D). Although the average length of the trout in the catch has increased somewhat, the average weight has almost doubled (Fig. 10E). There has been a marked decline in the percentage of mature trout in the catch, particularly since the mid-1950's (Fig. 10F). Conversely, the more recent fishery is exploiting about 25% more of the immature stock.

Many of these trends in the fishery began in the mid-1950's, when the effects of dietary changes were first expressed in the trout themselves. The increase in poundage harvested, the improvement in the catch per hour and per acre, the increase in the average weight of the trout caught, and the change in the proportions of mature and immature fish in the catch, can be related, at least in part, to the food changes in the late 1940's and early 1950's.

DISCUSSION AND CONCLUSIONS

An earlier study on plankton and fish-feeding lake trout in other Algonquin Park lakes (Martin, 1966) emphasized the importance of forage on the biology and dynamics of lake trout. Plankton-feeding trout grow slowly, are shorter lived, and mature at a smaller size and younger age than do fish-feeders. The fisheries in plankton-feeding populations provide large numbers of smaller and younger fish, a higher percentage of mature fish, and depend on fewer year-classes. It has also been shown (Martin and Sandercock, 1967) that a plankton diet may modify the structure of the trout by producing a greater number and more highly developed gill rakers.

The present investigation in Lake Opeongo has shown that diet changes within a single lake may have similar results. There appears to be a diet deficiency during certain periods in the life of the lake trout in Algonquin Park lakes. Growth curves, for example, show depressions (see Martin, 1952) at a time when lake trout may be changing from an invertebrate and small fish diet to larger food elements. Similar depressions are evident in the early years in the weight, growth, and gonad data in the present paper (Fig. 5–9). The food changes in Lake Opeongo have tended to eliminate these depressions, the cisco in particular serving to bridge this nutritional deficiency.

In drawing up a balance sheet of the effects of the changes that have occurred in Lake Opeongo lake trout, it is evident some may be considered detrimental, others beneficial. On the debit side is the fact the lake trout now mature later. The late maturity of this species has generally been considered to be one of its main weaknesses as an exploitable game fish. A higher percentage of immature fish are now being taken by the fishery before they have had the opportunity to spawn at least once. The virtual disappearance of the perch as a forage species, particularly for small lake trout, has apparently adversely affected the weight, growth, and gonad development of these fish. The disappearance of perch may also be a causative factor in the delayed maturity now found in the lake trout. It is possible that because of the greater abundance of food now available to the trout they may be more difficult to catch although this cannot be verified from the available information. For an angler desiring more lake trout rather than fewer but larger trout, the fishery may not be as attractive. However, there are many lakes in the same general area containing plankton-feeding trout in which such an angler can take large numbers of more readily available small fish (Martin, 1966).

On the credit side is the fact that the lake trout are now growing more quickly and are generally better conditioned. Egg production is now greater, particularly in the medium-sized fish that may contribute a high proportion of the eggs deposited on the spawning beds. The eggs are larger and perhaps hardier. The virtual disappearance of infertile fish should result in a substantially greater number of spawners and eggs available to reproduction. The fishery should be more attractive to anglers willing to spend more time to catch larger fish. Size has always been one of the lake trout's more desirable characters as a game fish.

Most of the year-classes from the last 10 years have only partially passed through the fishery and no assessment of their strengths can be made at this point. It will be of interest to see what effects, if any, the changes in maturity and fecundity will have on year-class production.

This study has emphasized the plasticity of the lake trout. It is quite possible that in future further external or internal pressures may again change the biological character of the trout in Lake Opeongo with subsequent effects on the fishery.

ACKNOWLEDGMENTS

The author is grateful to K. H. Loftus, Ontario Department of Lands and Forests, F. E. J. Fry, University of Toronto, and N. S. Baldwin, Great Lakes Fishery Commission, for their generous counsel and their time spent reviewing the manuscript.

REFERENCES

ALM, G. 1959. Connection between maturity, size, and age in fishes. Rep. Inst. Freshwater Res. Drottningholm 40: 145 p.

ATWOOD, A. D. 1960. Nutrition and reproductive capacity in fish. Proc. Nutr. Soc. 19: 23–28.

ESCHMEYER, P. H. 1954. The reproduction of lake trout in southern Lake Superior. Trans. Amer. Fish. Soc. 84: 47–74.

FRANZ, T. C., AND A. J. CORDONE. 1965. Introduction of the Bonneville cisco (*Prosopium gemmiferum* Snyder) into Lake Tahoe, California and Nevada. Calif. Fish Game 51: 270–275.

FRY, F. E. J. 1939. The position of fish and other higher animals in the economy of lakes. Amer. Ass. Advan. Sci. Publ. 10: 132–142.

1949. Statistics of a lake trout fishery. Biometrics 5: 27–67.

FRY, F. E. J., AND W. A. Kennedy. 1937. Report on the 1936 lake trout investigation, Lake Opeongo, Ontario. Univ. Toronto Stud. 42, Pub. Ont. Fish. Res. Lab. 54: 20 p.

HANSON, J. A., AND A. J. CORDONE. 1967. Age and growth of lake trout, *Salvelinus namaycush* (Walbaum), in Lake Tahoe. Calif. Fish Game 53: 68–87.

HANSON, J. A., AND R. H. WICKWIRE. 1967. Fecundity and age at maturity of lake trout, *Salvelinus namaycush* (Walbaum), in Lake Tahoe. Calif. Fish Game 53: 154–164.

HENDERSON, N. E. 1963. Extent of atresia in maturing ovaries of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). J. Fish. Res. Bd. Canada 20: 898–908.

MARTIN, N. V. 1952. A study of the lake trout, *Salvelinus namaycush*, in two Algonquin Park, Ontario, lakes. Trans. Amer. Fish. Soc. 81: 111–137.

1954. Catch and winter food of lake trout in certain Algonquin Park lakes. J. Fish. Res. Bd. Canada 11: 5–10.

1966. The significance of food habits in the biology, exploitation, and management of Algonquin Park, Ontario, lake trout. Trans. Amer. Fish. Soc. 95: 415–422.

MARTIN, N. V., AND F. K. SANDERCOCK. 1967. Pyloric caeca and gill raker development in lake trout, *Salvelinus namaycush*, in Algonquin Park, Ontario. J. Fish. Res. Bd. Canada 24: 965–974.

MCCAIG, R. S., AND J. W. MULLAN. 1960. Growth of eight species of fishes in Quabbin Reservoir, Massachusetts, in relation to age of reservoir and introduction of smelt. Trans. Amer. Fish. Soc. 89: 27–31.

SCOTT, D. P. 1962. Effect of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Bd. Canada 19: 715–731.

VLADYKOV, V. D. 1956. Fecundity of wild speckled trout (*Salvelinus fontinalis*) in Quebec lakes. J. Fish. Res. Bd. Canada 13: 799–841.

NOTES

The Species Problem within *Gasterosteus aculeatus* on the Pacific Coast of North America

D. W. HAGEN

College of Fisheries
University of Washington, Seattle, Wash., USA

AND

J. D. MCPHAIL

Department of Zoology
University of British Columbia, Vancouver, B.C.

HAGEN, D. W., AND J. D. MCPHAIL. 1970. The species problem within *Gasterosteus aculeatus* on the Pacific coast of North America. J. Fish. Res. Bd. Canada 27: 147–155.

Two kinds of *Gasterosteus aculeatus, leiurus* and *trachurus*, are considered species in several streams and lakes in British Columbia. The two sticklebacks may act as species over a much wider range, but at present there is insufficient evidence for firm conclusions. The data of Miller and Hubbs (Copeia 1969: 52–69, 1969) given as evidence for introgression between *leiurus* and *trachurus* on the Pacific coast cannot be taken as well established. Should conclusive data for introgression be forthcoming, it would then seem that the two sticklebacks act as species in places and as subspecies in others. At least along the Pacific coast the pattern of variation found within *Gasterosteus* is exceedingly complex and cannot be accounted for wholly upon any simple explanation in terms of hybridization between *leiurus* and *trachurus*. There is evidence that much of the interpopulation variation is a result of adaptation and natural selection — not introgression. It will in fact require much more investigation before we can proclaim the causes of the variation we see so strikingly in *Gasterosteus*.

Received September 9, 1969

AS THE RESULT of an experimental study of isolating mechanisms between two kinds of *Gasterosteus aculeatus* in the Little Campbell River, British Columbia, Hagen (1967, p. 1638) concluded as follows: "*Leiurus* and *trachurus* are reproductively isolated, have well developed isolating mechanisms, and exhibit considerable genetic divergence. The two, then, fulfill the species definition of Mayr." And then, "Reproductive isolation between *leiurus* and *trachurus* seems to be widespread, throughout their range." Miller and Hubbs (1969) take exception to these conclusions and give data that they hold as

Printed in Canada (J1587)

evidence that *leiurus* and *trachurus* should be regarded as subspecies. We are glad to take this opportunity to discuss the species problem within *G. aculeatus*.

Hagen considered *leiurus* and *trachurus* as species in the Little Campbell River for the following reasons. First, ecological isolation between the two is strongly developed. Each selects different habitats in which to breed. The habitats differ in a number of characteristics such as current, vegetation, substrate, and water quality. Preference tests in the laboratory and transfer experiments in the stream demonstrated the effectiveness of ecological isolation. Ecological isolation was also examined in four streams on Vancouver Island, and in all instances where the two sticklebacks were sympatric within a stream they were found segregated into the same kinds of habitats as those in the Little Campbell. For example, in the Big Qualicum River *leiurus* is confined to densely vegetated marshes that fringe the lower portions of the stream whereas *trachurus* breeds in the headwaters 7 miles inland. Although the two are segregated into the same kinds of habitats as those in the Little Campbell, the pattern is reversed. That is to say, in the Little Campbell *trachurus* breeds in the lower portion of the stream, *leiurus* in the headwaters. If these facts are considered along with the preference tests and transfer experiments, the only explanation would seem to be differences in response to environmental cues by *leiurus* and *trachurus*. Ecological isolation appears to be well developed.

Second, seasonal isolation is partially developed, and there is some evidence this difference has a genetic basis. Evidence for the latter is not good.

The best reason for considering *leiurus* and *trachurus* as species is based upon natural selection. *Leiurus* and *trachurus* hybridize in very narrow zones in the Little Campbell and in Bonsall Creek (1 mile and 200 ft, respectively) where their habitats are contiguous. Breeding experiments in the laboratory (F_1 hybrids, backcrosses, and parental types) demonstrated considerable genetic divergence between the sticklebacks. Since hybrids are confined to very narrow zones, and there are considerable genetic differences between them, natural selection must be blocking gene flow very effectively. Large populations of *leiurus* and *trachurus* exist either side of these narrow zones. There is no evidence from any of the characters analyzed that introgression occurs. Indeed, the presence of a reversed cline for lateral plates (shown to be a genetic trait) in *leiurus*, becoming progressively more distinct from *trachurus* as the hybrid zone is approached, is strong evidence against introgression and indicates severe selection counteracting genetic exchange. Furthermore, transfer experiments demonstrated that when each stickleback was displaced to the habitat of the other, they either abandoned the habitat, or died. This again is taken as evidence for the effectiveness of natural selection. The indirect evidence for selection against hybrids, existence of isolating mechanisms, very narrow hybrid zones, lack of introgression, reversed clines, and transfer experiments all point to the same conclusion; *leiurus* and *trachurus* are reproductively isolated. They are species as defined by Mayr (1963).

We now know that in lakes Kennedy and Stave, British Columbia, *leiurus* and *trachurus* coexist permanently together. They are isolated within these

large lakes by impassible falls or dams, and in both lakes the frequency of hybrids is less than 10%. Here too, selection must be successfully eliminating hybrids such that the two sticklebacks remain sympatric and distinct, as species. Moreover, Hay (MS, 1968) has found considerable ethological isolation between *leiurus* and *trachurus*, such that females show choice during courtship for conspecific males.

There is evidence in the literature that the situation in the Little Campbell River and on Vancouver Island may be widespread. Heuts (1947, p. 90–91) who worked with *G. aculeatus* in Belgium, states: "Individuals intermediate between these modes do occur, but they are not frequent in any population." And later, "Within a given drainage, the two types may approach one another but scarcely mix." He shows data for the distribution of the two types in streams, and the relationships of the two sticklebacks and of narrow zones with intermediates is remarkably like that in the Little Campbell.

If, on the other hand, there is evidence elsewhere for introgression as Miller and Hubbs claim, the only reasonable conclusion would seem to be that *leiurus* and *trachurus* act as species in places and as subspecies in others. We would then have another instance very much like the two towhees described by Sibley (1961).

Miller and Hubbs (p. 53) first admit that any decision as to the species status is largely arbitrary in those instances concerning hybrid zones. They then proceed to the conclusion that *leiurus* and *trachurus* are not species. Hagen (p. 1688) pointed out that Sibley (1961) and Bigelow (1965) have given special attention to the problem of how one is to interpret the species status in instances of hybrid zones. If there is evidence for selection against hybrids, for considerable genetic differences, and evidence that reproductive isolation (genetic isolation) is maintained by both parental types on either side of the zone, then the two are considered species (see above papers for a full discussion). This is the situation in the Little Campbell. We accept these criteria of Sibley and Bigelow because they are biologically meaningful, and they give us objective criteria for our decisions. Thus, we would not agree with Miller and Hubbs when they say such instances are subjective and arbitrary. Many species are known to interbreed (Sibley, 1961; Mayr, 1963; Remington, 1968) yet selection maintains reproductive isolation.

Miller and Hubbs give two reasons for their contention that *leiurus* and *trachurus* should be regarded as subspecies. First, they say, "One reason for maintaining the species intact is that this is the conservative course, preserving long-established practice." This seems a very dubious basis for procedure in taxonomy. The history of taxonomy is full of instances in which "long-established practice" had to be abandoned because subsequent investigation demonstrated the inadequacy of our earlier views or methods. All one need do to realize this is pick up any taxonomic revision or refer to any of the recent reviews concerning history of concepts or methods (for instance, Cain, 1959, 1962; Mayr, 1968).

Their second reason for considering *leiurus* and *trachurus* as subspecies is based upon their claim that introgression occurs. Under a section of their paper entitled "Analysis of Two Cases of Apparent Introgression Between the *Trachurus* and *Leiurus* Types of *G. aculeatus*," Miller and Hubbs present their data that they take as evidence for introgression. The evidence is based almost entirely upon preserved specimens, although ecological notes are included. The first case is based upon a sample of 1386 specimens that varied from 12 to 47 mm in standard length. Since individuals with low lateral plate counts were found, they interpret this as *trachurus* (high plate counts) modified by introgression from *leiurus* (low plate counts). However, this conclusion is open to serious objection. We have found (unpublished data) that if one counts lateral plates on specimens from 25 to 30 mm under a binocular dissecting scope (taking the precaution to dry the specimens), and then recounts plates after staining with alizarin dye, there is a discrepancy between the two methods of about 30%. The error arises because unless one does stain there are small delicate plates that are not counted. We find no such difference for plate counts of specimens above 30 mm, indicating that staining is not necessary for specimens above that size. The results were obtained from counts on more than 300 specimens. We also have data (to be published elsewhere) which indicates that number of plates on sticklebacks is not complete until they have reached a size of about 25 mm. Counts from specimens less than this size would again be wrong. The sample of Miller and Hubbs included specimens from 12 to 47 mm, and the only statement they make as regards this important point of methods is as follows: "Excluding specimens too small to show complete plate development. . . ." But this appears to be an arbitrary decision on their part. For both reasons given above the specimens with low plate counts may very well not be a result of introgression, but a result of wrongly made plate counts. The same objections apply to their second case of "introgression." Hence, their case for introgression is not well substantiated. Moreover, they themselves admit (p. 68) it is likely that individuals with lower plate counts may arise in populations of *trachurus* as a result of recombination rather than a result of introgression. Hagen gave some evidence that this was the case in a particular population, and it is that population to which Miller and Hubbs refer, and they agree that recombination is the more likely explanation. Thus, when individuals with low plate counts are found within populations of *trachurus*, we should be cautious in our interpretation, for there are at least two explanations for such individuals. We cannot state, categorically, that it is a result of introgression. Such individuals within *trachurus* populations may result from recombination within balanced polygenic complexes.

Although Miller and Hubbs relied heavily upon plate counts for their analysis of "introgression," they later (p. 68) say, "By considering several characters, analyses can be made more certain and more complete. For example, critical attention to other characters would have made it possible for Hagen to have identified more fully the range of variation and relative numbers of natural hybrids (intergrades), in Little Campbell River, by avoiding the cutoff

in number of lateral plates that corresponds to the hiatus between his counts for the allopatric populations."

We cannot guess how Miller and Hubbs reached this conclusion. Hagen states under Materials and Methods (p. 1643), "*Leiurus* and *trachurus* differ in a number of characters. Those chosen for analysis were number of lateral plates, number of gill rakers, electrophoretic patterns, standard length at sexual maturity, body depth into standard length at sexual maturity, and numbers of dorsal and anal rays." Clearly the purpose of the hybrid index was to compare *all* such characters in *leiurus* and *trachurus* against specimens from the hybrid zone. Also proportions of hybrids and parental types within the hybrid zone (based on all characters) are given (p. 1646, fig. 4; p. 1649, fig. 7). Furthermore, data are given for the distributions of *leiurus*, *trachurus*, and hybrids (based on all characters but electrophoretic patterns) throughout the length of the stream (p. 1648–1649, fig. 7).

Miller and Hubbs interpret almost all the phenotypic variation we see in populations of *G. aculeatus* as a result of hybridization (intergradation) or introgression. For example, "More compelling is the evidence for intergradation and introgression between the different types,"; "Hagen failed to state that much data on intergradation,...,"; "...definitely seem to represent *trachurus* extensively modified by past hybridization with *leiurus*, but reintegrated by inbreeding,"; "Past hybridization and subsequent mass backcrossing to the *leiurus*...,"; "...very likely as a result of introgression,"; "...comprise an introgressed population...,"; "...some admixture and probable hybridization with *trachurus* occurs,"; "...seem to comprise an unintegrated mixture of the *trachurus* type (*G. a. aculeatus*) and the *leiurus* type (*G. a. microcephalus*) other collections appear to show intergradation and introgression,"; "The conclusion again is that the population sampled has been integrated following some past hybridization," and so on.

There are at least two possible explanations for the phenotypic variation we see in *Gasterosteus* but Miller and Hubbs, seemingly preoccupied with introgression and hybridization, make no mention of one. The other explanation is adaptation and natural selection. If there is evidence that the characters used to distinguish *leiurus* and *trachurus* are adaptive, then it is likely that the particular complex of characters and their mode of variation is a result of natural selection. For instance, Miller and Hubbs imply (p. 57) that in southern California where there is no contact with *trachurus*, plate counts of freshwater populations are unusually low or even absent because of no opportunity for introgression. But further north where *trachurus* occurs plate counts of *leiurus* are higher and as they say, "very likely as a result of introgression." This explanation is not adequate. One of us (J.D.M.) is now working with a population of plateless sticklebacks on Texada Island, off the coast of British Columbia. This is well within the range of *trachurus* and there is ample opportunity on this small island for introgression. It is likely that selection favours and maintains plateless forms in this lake irrespective of any hybridization. There are similar populations of plateless sticklebacks in the

Queen Charlotte Islands (E. E. Moodie, personal communication). The situation in the Little Campbell, Bonsall Creek, Stave Lake, and Kennedy Lake clearly demonstrates that natural selection can effectively prevent introgression from altering the phenotypes of *leiurus* or *trachurus*, given the constant opportunity for doing so. This is the case for all characters investigated but is especially clear for lateral plates that show reversed clines, with constant hybridization. Selection must be most effective in holding the two phenotypes at their respective values.

Now McPhail, Moodie, and Hagen have independently obtained further evidence (to be published elsewhere) that plates are subject to strong selection. One of us (D.W.H.) has data for samples taken from over 40 freshwater localities in Washington and British Columbia. The taxonomic characters treated include plates, gill rakers, and vertebrae. If one pays strict regards to ecology, there exists correlations between environment and frequency distributions for such traits. For instance, there are very large modes (50–70% of the population) at seven plates showing high correlations with respect to certain environmental factors. This suggests straight away such traits (shown to be genetic) are subject to strong environmental selective agents. But Miller and Hubbs interpret such populations as due to introgression (p. 57). We and Moodie have evidence from laboratory experiments and from natural populations that differential predation may be one of the selective forces favouring seven-plated fishes where predation is intense. Furthermore, Moodie (MS, 1968) has good evidence that seven-plated sticklebacks show significant differences from six- and eight-plated ones with respect to cover when choosing nesting sites. McPhail has similar evidence for another population on Texada Island. As mentioned, other taxonomic traits show correlations with environment. For example, gill-raker numbers in lakes are higher (an adaptation to planktonic feeding?) than gill-raker numbers from streams, and similar correlations hold for vertebrae. It would then seem that selection favours individuals with higher rakers in lakes than in streams within *leiurus* and the same is true for vertebrae. Therefore, in lakes as opposed to streams *leiurus* shows convergence upon *trachurus* (which has higher gill-raker counts).

There is a population of *Gasterosteus* in Quinault Lake, Washington, showing pecularities for several characters. In this large lake virtually all fish have plate counts intermediate between those of *leiurus* and *trachurus*, having anterior plates, a caudal keel of plates, and a gap without plates between (sample of 450). Gill rakers are also higher than usual for stream-dwelling *leiurus*, so they are intermediate between *leiurus* from streams and *trachurus*. Some would take this as indicating past introgression. But there is only one known force that can hold such a phenotype (genotype) stable in a population that must number in the thousands, and this is selection. If intermediates were not being selected for, plate number would vary enormously. It is clear that natural selection must favour such an intermediate phenotype in Lake Quinault, and since there is evidence that these taxonomic characters are adaptive and subject to strong selection, the population in Lake Quinault

may have evolved from *trachurus* or from *leiurus*, without any past hybridization. Granting the great phenotypic and genetic flexibility we find in *Gasterosteus*, there must be adequate variation for such adaptive evolution to occur, and with strong selection it may occur quite rapidly. In other words, convergence is a very real problem.

There are further reasons for accepting this contention (introgression) of Miller and Hubbs meekly. They say, "These counts supplement the generally neglected but extensive data of Rutter (1896, p. 248–249) for the whole Pacific Coast. Rutter's data and ours, for the well-watered coastal region of California from San Francisco Bay northward, as well as of Oregon and Washington, indicate that the *trachurus* type (which we have been calling *G. a. aculeatus*), or a stock grading more or less toward *leiurus* (our *G. a. microcephalus* Girard), seemingly as a result of introgression, occupies most of the streams sampled, with or without a proportion of *leiurus* and/or *semiarmatus*."

First, this implies that the Pacific coast has been extensively sampled for *Gasterosteus*. Second, it indicates a pattern of variation made up of *leiurus*, *trachurus*, and introgression between the two. We have found a population of uniquely large, sooty-black sticklebacks in which nuptial colours of most males are black. They are endemic to Mayer Lake, Queen Charlotte Islands, and as mentioned are the object of a Ph.D. thesis (Moodie, MS, 1968). On Texada Island, British Columbia, one of us (J.D.M.) has found a population of sticklebacks with no plates, no pelvic girdle, no pelvic spines, rudimentary dorsal spines, and other pecularities. Moodie has found similar populations in the Queen Charlotte Islands. On Texada Island there is a population of *Gasterosteus* that superficially resembles *Pungitius pungitius*. They have an astonishingly terete body, a long threadlike caudal peduncle, and gill-raker counts that far exceed other known populations. Other populations in these islands and in the Queen Charlottes are two-spined (dorsal spines) or are polymorphic for spine number. In the vicinity of Ocean City, Washington, we are working with populations where males have jet-black breeding colours that can be "turned off" within seconds, and have other unusual traits (McPhail, 1969). In Quinault Lake we have found a population in which more than 90% of the fish have a caudal keel, anterior plates, and a gap with no plates between. Males have orange throats at breeding time. In lakes Chelan, Roses, and Wapato, Washington, there are populations having large modes (about 60% of the fish) at seven plates, and less than 5% with plate counts near 30. These populations are "dwarfed" with respect to *leiurus* or *trachurus* and male nuptial colours may be black beneath, nonexistent, red-throated, or entirely blue. In Stave Lake and Kennedy Lake, British Columbia, *leiurus* and *trachurus* are landlocked and coexist sympatrically. In many streams along the Columbia River, Fraser River, and on Vancouver Island, there are landlocked populations of *trachurus*.

We cannot claim to have collected the Pacific Northwest at all extensively, and it is highly unlikely we have just happened across these and other such unusual populations of *Gasterosteus*. It is far more likely the Pacific Northwest

has never been "extensively" sampled for sticklebacks, as Miller and Hubbs imply. And the pattern of variation is far more complex than we would have ever dared to guess. Any simple explanation wholly in terms of hybridization between *leiurus* and *trachurus* seems most untenable. Although not denying that introgression may occur, it will in fact require much more investigation before we can proclaim the causes of the phenotypic variation so strikingly shown in *Gasterosteus*.

There is no need for surprise that the taxonomic characters used to define *Gasterosteus* are so variable from population to population. The criterion of nonadaptive (neutral) characters has been abandoned. It now seems that all such characters are adaptive (Cain, 1959; Mayr, 1968, for reviews). Nor need we show surprise that such characters may be subject to intense selection showing striking changes in space, or time. The review by Ford (1964) opens our eyes to the sorts of selection coefficients we may expect to find in natural populations.

Miller and Hubbs dislike our referring to the Pacific Ocean populations as *leiurus* and *trachurus*. They say, "Whether the Pacific Ocean representatives of the *leiurus* and *trachurus* types should be separated from those of eastern North America and/or those from Europe is still subject to research, which might well include studies of chromosomes, immunological reactions, mating experiments,..." and they go on to suggest the matter will be settled after a detailed comparison of such taxonomic characters is made. For that matter, we do not know that the fish we call *G. aculeatus* in Europe is the same species as the one we call *G. aculeatus* on the Pacific coast. For all we know hybrids may be completely sterile. Yet Miller and Hubbs do not object to calling both *G. aculeatus*. Equally we do not object to calling Pacific coast populations *leiurus* and *trachurus* in keeping with European practice. In either case a revision of nomenclature may be necessary *once the data are in hand.*

Furthermore, Miller and Hubbs take "strong stand" against not recognizing subspecies. Although agreeing that subspecies could be useful, we do not feel so strongly about the matter. Difficulties with subspecies have very often been exposed (see Sokal, 1965; Mayr, 1968). Cain (1959) puts the problem very well when he says,' "At the level of the subspecies, there has been much trouble and many discussions about the amount of difference necessary before a population should be given a subspecific name. Some have given names on the slightest perceptible difference (some, indeed on less) while others, not in the least denying the existence of these slight differences, have felt that there was no point in their receiving nomenclatorial recognition. How different must one subspecies be, then, from its nearest relative?" And later, "It is a matter for arbitrary definition and agreement among taxonomists. This may seem a small thing, but we have only to think of the usefulness of that well-known object in Paris, the standard metre, to see what arbitrary agreement may lead to." Until such agreement is reached there will continue to be difficulties. If one were not cautious with his use of the subspecies in *Gasterosteus*, a flood

of names could easily follow, given the amazing interpopulation variation we find.

One of the great merits of the biological species definition, so soundly hammered home by, for example, Mayr (1963), is its objectivity. Here we do have more objective criteria than, for example, the completely arbitrary one (sometimes called the "eyeball" technique) often used in the past. The biological species concept together with the more sophisticated techniques of numerical taxonomy are sure to advance the field. The more objective our criteria, this is pure gain for science, including taxonomy. Surely there are instances where the biological species definition is not rigorously operational. Nevertheless there is no excuse for not applying the definition where we can, as with *Gasterosteus*. In such instances subjective criteria are gone forever.

Acknowledgments — We are grateful to Mr E. E. Moodie for calling an important point to our attention, and to Messrs Moodie and D. Hay for allowing us to quote some findings from their research. One of us (D.W.H.) acknowledges support for his research, NSF Grant No. 7686.

REFERENCES

BIGELOW, R. S. 1965. Hybrid zones and reproductive isolation. Evolution 19: 449–458.

CAIN, A. J. 1959. Taxonomic concepts. Ibis 101: 302–318.
1962. The evolution of taxonomic principles. *In* G. G. Aimsworth and P. H. A. Sneath [ed.] Microbial classification. 12th Symp. Soc. Gen. Microbiol., Cambridge Univ. Press, London.

FORD, E. B. 1964. Ecological genetics. John Wiley and Sons, Inc., New York.

HAGEN, D. W. 1967. Isolating mechanisms in threespine sticklebacks (*Gasterosteus*). J. Fish. Res. Bd. Canada 24: 1637–1692.

HAY, D. MS, 1968. M.Sc. Thesis. University of British Columbia, Vancouver, B.C.

HEUTS, M. J. 1947. Experimental studies on adaptive evolution in *Gasterosteus aculeatus* L. Evolution 1: 89–102.

MAYR, E. 1963. Animal species and evolution. The Belknap Press, Harvard Univ. Press, Cambridge, Mass.
1968. Principles of systematic zoology. McGraw-Hill Book Co., New York.

MCPHAIL, J. D. 1969. Predation and the evolution of a stickleback (*Gasterosteus*). J. Fish. Res. Bd. Canada 26: 3183–3208.

MILLER, R. R., AND C. HUBBS. 1969. Systematics of *Gasterosteus aculeatus*, with particular reference to intergradation and introgression along the Pacific coast of North America: a commentary on a recent contribution. Copeia 1969: 52–69.

MOODIE, E. E. MS, 1968. Ph.D. Thesis. University of Alberta, Edmonton, Alta.

REMINGTON, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. Evol. Biol. 2: 321–428.

SIBLEY, C. G. 1961. Hybridization and isolating mechanisms, p. 69–88. *In* W. F. Blair [ed.] Vertebrate speciation. Univ. Texas Press, Austin, Texas.

SOKAL, R. R. 1965. Statistical methods in systematics. Biol. Rev. 40: 337–391.

Three Computer Programs: Back-Calculation, Condition Factor, and Stomach Content, CDC 3600 Fortran/Format

J. C. MAWSON AND R. J. REED

Department of Forestry and Wildlife Management
University of Massachusetts, Amherst, Mass. 01002, USA

MAWSON, J. C., AND R. J. REED. 1970. Three computer programs: back-calculation, condition factor, and stomach content, CDC 3600 Fortran/format. J. Fish. Res. Bd. Canada 27: 156–157.

Three programs were developed to be used on the CDC 3600 computer at the University of Massachusetts: back-calculation, condition factor, and stomach content. They are written in standard Fortran IV notation, which can be adapted to any computer setup that accepts Fortran IV and has at least the storage capacity listed for each program. Instruction sheets and sample output can be secured from either author.

Received August 5, 1969

THE WIDESPREAD USE of computer programs in the analysis of fisheries data is now a necessity. The three computer programs described in this article can quickly calculate data useful to the fishery biologist in both the management and research fields.

Availability — Instruction sheets and sample output on all these programs can be secured from either author. The BACKCAL and KFACTOR programs have been adapted to the UMASS teletype system and these listings are also available.

Program BACKCAL

This program is designed to back-calculate the length at each annulus from scale measurements and fish length at capture. A linear body-scale relationship is assumed. Calculations are based on a constant intercept ("a" value) or correction factor. The slope (b) is also calculated for each fish $\left(\frac{\text{total length} - a}{\text{scale radius}}\right)$ for a single sampling year.

The program performs calculations and prints output as follows: (1) b value (slope) for each fish. (2) Length at each annulus per fish. (3) Average length of each annulus by age class and sex (if recorded); otherwise, the entire sample is combined. (4) Absolute growth and annular increments by age class. (5) Number of fish recorded by year and age class.

Control options permit selection of desired output using different intercepts and either separates by sex or gives averages for all fish combined.

Printed in Canada (J1551)

Storage — The current program uses 16,684 storage units.

Restrictions — The program is limited to 300 fish and a maximum of 10-year-old fish. A single batch run on the University of Massachusetts CDC 3600 computer would permit an increase to 400 fish. Other computers with comparable storage configurations may permit even a greater dimension on the number of fish or age classes.

Program KFACTOR

This program calculates condition (K) based on the equation

$$K = \frac{W \times 10^5}{L^3}$$

where W = weight in grams and L = length in millimeters. The program performs this calculation and prints output as follows: (1) Identification number, length, weight, and condition factor for each fish. (2) Average condition by sex (male, female, immature) if recorded; plus the grand average condition factor for all fish. (3) If the fish have not been sexed, the statement "no fish in this class" will be printed in place of the male, female, and immature data. Then the grand average condition factor for all fish is computed and printed.

Storage — The current program uses 8128 storage units.

Restrictions — The current program is limited to 300 fish but certain dimension statements can be changed to increase this number to 1000 fish, if necessary.

Program STOMACH

This program performs calculations and prints output as follows: (1) Identification number, age, and with 100% the total for an individual fish, the percentage frequency of occurrence of each food item found in that fish. (2) The percentage of stomachs in which a food item occurs. (3) Number of fish in the sample.

Control options permit changing the 15 food-item categories to match stomach content data. Those categories not used produce zero output and remain blank on the data cards. Your sample must be sorted by age classes or other definitions if required. Percentages will be derived for each group and a grand average calculated for the entire sample if this sort is made.

Storage — The program uses 7658 storage units.

Restrictions — The number of fish per sample is limited to 300 but this could be increased to 1100 if minor changes are made in the dimension statements.

Fish Chromosome Preparation: Air-Dried Displays of Cultured Ovarian Cells in Two Killifishes (*Fundulus*)

T. R. CHEN

Department of Biology, Kline Biology Tower
Yale University, New Haven, Connecticut, USA

CHEN, T. R. 1970. Fish chromosome preparation: air-dried displays of cultured ovarian cells in two killifishes (*Fundulus*). J. Fish. Res. Bd. Canada 27: 158–161.

A relatively simple and reliable in vitro methodology for fish chromosome study is described. A single cell suspension obtained by trypsinizing ovarian tissue was cultured for 5–7 days at 31 C. The cells were treated with colchicine and hypotonic solutions then fixed in acetic–alcohol fixative. Air-dried preparations were made from these cells and were stained with aceto-orcein. Numerous metaphase plates unmarred by overlapping chromosomes were recovered and high quality karyograms were available for detailed karyotypic analysis. The general similarity of squash and air-dried karyotypes suggests that short-period cell culture preserves natural karyotypes.

Received September 23, 1969

MOST CHROMOSOMAL STUDIES of teleostean fishes have emanated from squash preparations whose techniques, which vary widely among investigators, include the uncontrollable but critical phases of accumulation of sufficient metaphase plates, proper hypotonic pretreatment, fixation, and subsequent squashing to avoid clumping of chromosomes and breaking of metaphase cells (Ohno, 1965; McPhail and Jones, 1966; Chen, MS, 1967). To standardize and improve preparations, recent developments in tissue culture method apply to cytogenetic studies of fishes, whose karyotypes comprise mostly small chromosomes. Roberts (1964) derived squashed karyotypes of centrarchids from primary cell cultures and Levan et al. (1966) of the fathead minnow from a permanent cell line. Regan et al. (1968) and Chiarelli et al. (1969) first used the air-dried technique to display cultured fish cells for chromosome study.

The present report introduces a relatively simple and reliable method for culturing fish cells and preparing air-dried karyotypes. It improves on other methods in that (1) more well-spread metaphase plates unmarred by overlapping chromosomes are recovered; (2) fewer cells are broken if preparations are properly handled; and (3) fixation and staining are consistently excellent.

Method — Of the two local killifishes studied, *Fundulus majalis* is restricted to salt and brackish waters, and euryhaline *F. heteroclitus* occurs widely in salt and fresh waters. Both species were collected from salt water and kept in aquaria for 1–3 months before the experiments. To test their reliability, the culture and air-dry procedures were repeated once for each species within 3 weeks, totaling four experiments. Females were removed from saltwater aquaria, rinsed briefly in tap water, killed by severing their spinal cord, and surface-sterilized with absolute ethanol. All dissecting instruments were sterilized by alcohol dipping and flaming before all operations.

Printed in Canada (J1595)

Short-term cell culture — The extirpated ovaries were placed into 5 ml of 0.25% viokase (Gibco trypsin product) in a tissue culture dish and minced with crossed knives. The fragments underwent further dissolution in 10 ml of viokase at room temperature on magnetic stirrer for 15 min, when action was stopped with 1.0 ml of Fetal Bovine Serum (FBS), increasing the concentration to 10% serum. Cells were spun down, collected, and resuspended with 1 ml of F-10 (or F-12) culture medium and then innoculated into a 30-ml Falcon culture flask prefilled with 4 ml of the medium and gassed with a mixture of 5% CO_2–95% air. This flask was then incubated at 31 C for 5–7 days with one feeding (expedited after 3–4 days of incubation by removing 2 ml of old medium from the bottle and refilled with equal amount of new.) After 5–7 days, enough cells were recovered for making preparations.

Chromosome preparations — Six to 8 hr before recovering cells, 0.05 ml of 100 μg/ml colcemid solution (100 μg colcemid dissolved in 1 ml double distilled water) was added to the culture flask to bring the final concentration to 1 μg/ml. After decanting the medium, 3 ml of hypotonic trypsin and versene solution was added and, after incubating for 10 min, the resulting suspension was collected in a centrifuge tube prefilled with 0.3 ml FBS to decrease the concentration of hypo-TV to 10% serum. Clumps or attached cells were dispersed by gently pipetting the suspension over the flask bottom. If necessary to remove the remaining cells in the bottle, the suspension can be replaced with 3 ml of fresh hypo-TV.

The preparation was then spun down at 1000 rpm for 10 min, decanted to leave 0.5 ml, resuspended, treated with 4 ml of 0.075 M KCl (hypotonic) solution added slowly with continuous stirring, incubated for 10 min, and centrifuged again at the same speed.

Supernatant was again decanted, leaving about 0.5 ml for resuspending pellets, before freshly prepared acetic–alcohol fixative (1 part glacial acetic acid to 3 parts absolute methanol) was added, slowly with continuous stirring, to increase the total volume to 4–5 ml. This was left for 15 min before spinning down at the same speed.

As much supernatant as possible was then removed without disturbing the pellets, so that fresh fixative, added as before, replaced the old. Again, a 15-min wait preceded centrifugation.

The pellets were resuspended in the supernatant, which measured about 10–15 times the volume of the pellets. About three drops of the suspension placed on a chilled wet slide were distributed by gentle blowing, air-dried quickly, and left overnight before staining.

The air-dried slides were immersed in freshly filtered aceto-orcein (stock solution: 1.5 g of Gurr's synthetic orcein in 100 ml of 65% acetic acid) for 15 min. After excess stain was removed in absolute alcohol, the stained slides were mounted under #1 cover slips in Canada balsam.

Karyotypes of both species were also studied employing squash technique that was described elsewhere (Chen, MS, 1967; Chen and Ebeling, 1966, 1968). In brief, colchicinized gill epithelium, spleen, kidney, and testis in vivo were pretreated with 0.9% sodium citrate solution and directly squashed in aceto-orcein or squashed in aceto-orcein after being fixed in 1:3 acetic alcohol. Observations were carried out from these temporary preparations sealed with Kronig cement.

All preparations were examined under phase contrast optics and photographed with Zeiss photomicroscopy using kodak high contrast copy film.

Results — Previous squashes showed that both species have $2n = 48$ chromosomes, with no noticeable inter- or intra-individual chromosomal polymorphism (Chen and Ruddle, 1970). From the present air-dried preparations, 60–80 metaphase plates in each of four experiments showed no more than two chromosomal overlaps per cell, most chromosomes more or less symmetrically dispersed, and less than 6% aneuploidy (Table 1). Specifically, 30–40% of the metaphase plates had no chromosome overlaps and 94% had $2n = 48$ counts, showing no karyotypic variability and indicating that most cells remain intact for karyotype analysis. Moreover, they had not noticeably

TABLE 1. Diploid chromosome counts of *Fundulus majalis* and *F. heteroclitus* from short-period ovarian cell culture.

Species	Expt.	N (metaphase plates)	Chromosome counts per cell				% aneuploidy
			46	47	48	49	
F. majalis	1	80	1		76	3	5.0
	2	60	2		57	1	5.0
	1 & 2	140	3		133	4	5.0
F. heteroclitus	1	60		3	56	1	6.7
	2	60		1	57	2	5.0
	1 & 2	120		4	113	3	5.8

mutated during the week's culture, so that a relative abundance of presumably natural karyotypes were obtained.

Among hypoploid cells, all chromosomes resembled the normal karyotypes, i.e., neither additional biarmed nor distinctly large or small chromosomes indicated possible chromosomal interchanges. In the hyperploid cells, however, one cell of *F. heteroclitus* and two of *F. majalis* contained an additional acentric chromosome fragment. Because the meaning of these hyperploid counts (49) was unclear, they were simply lumped together as different from those of the normal karyotype with 48 chromosomes. Except for a single apparently tetraploid count, no cells exceeded 49 chromosomes.

In general, squash and air-dried preparations produced similar karyotypes, except for the greater clarity and definitions of the air-dried, which, therefore, warranted the following morphological observations. In *F. heteroclitus* (Fig. 1), two chromosones included distinct satellites on their short arms, not easily identifiable in most squashes. In *F. majalis* (Fig. 2), two large chromosomes showed a distinct secondary constriction on the long arm near the kinetochore. The smallest submetacentric pair in the complement was satellited with relatively long stalks, so that their entire length approximated that of the longest acrocentrics. Although squash materials were mainly obtained from gill epithelium and spleen, whereas the air-dried were from ovaries, all karyotypes were apparently identical. Therefore, short-period culture and air dryings apparently yielded natural karyotypes.

Conclusions — Air-dried preparations are superior to squashes in that subtle characteristics of chromosome morphology, such as satellites and secondary constrictions are recognizable in most cells. So called aneuploid cells most likely result from loss or gain of chromosomes during handling and their low frequency (5–6%) does not create serious error. The general similarity of squash and air-dried karyotypes further suggests that short-period cell culture preserves natural karyotypes. Because replicated experiments yield almost identical karyotypes, cultured and air-dried preparations appear as or more reliable than in vivo squashes.

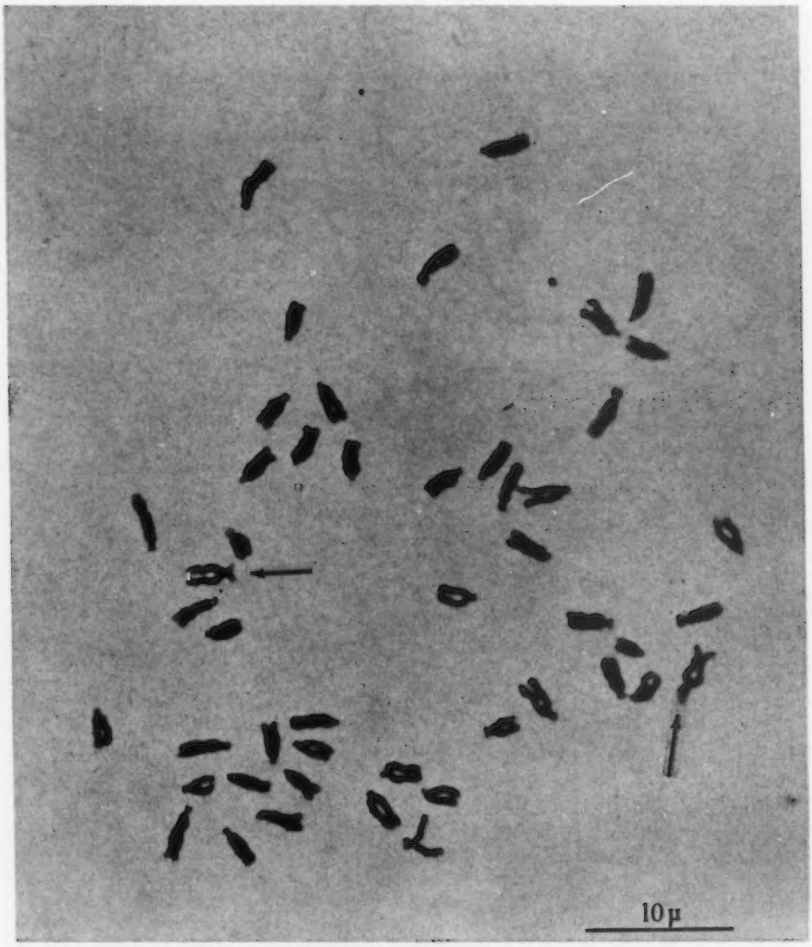

FIG. 1. Metaphase plate of *Fundulus heteroclitus* (air-dried preparation from ovarian cell culture, stained with aceto-orcein). Arrows show chromosomes with discernible satellites on their short arms (satellited chromosomes).

Chen — J. Fish. Res. Bd. Canada

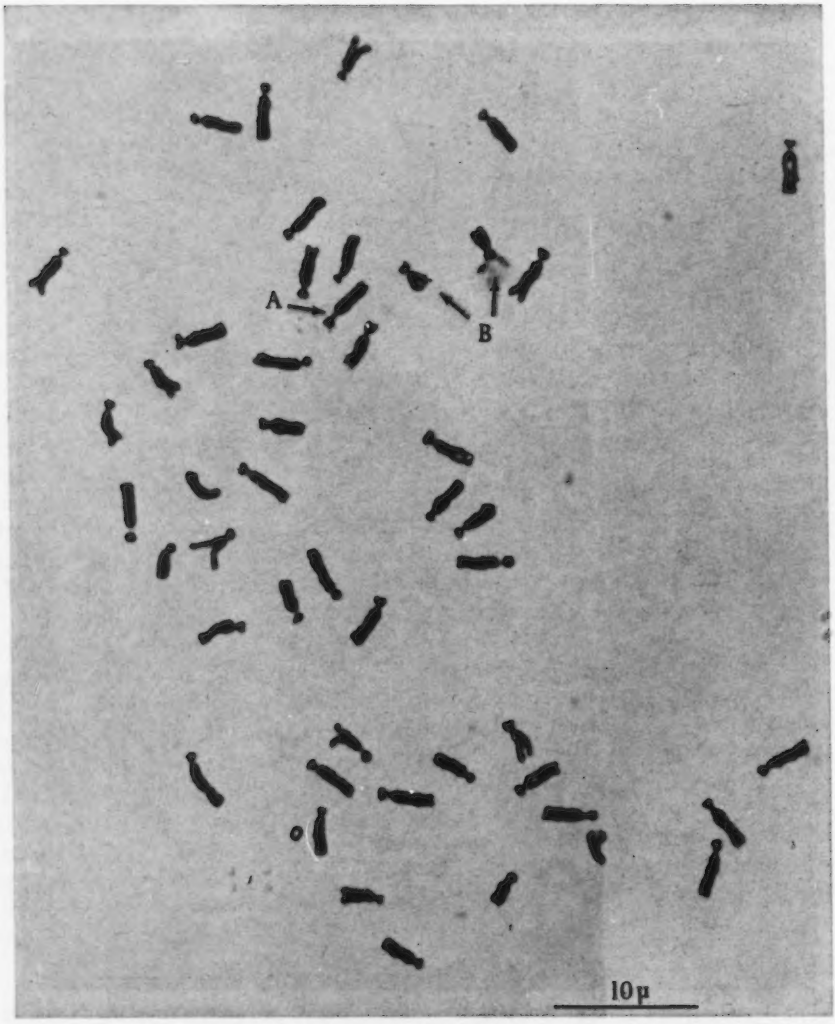

FIG. 2. Metaphase plate of *Fundulus majalis* (air-dried preparation from ovarian cell culture, stained with aceto-orcein). A, chromosome with secondary constriction in the long arm near the kinetochore (elongated constricted portion); B, satellited chromosomes.

Acknowledgments — I thank Mr R. J. Klebe (Yale University) for technical assistance in cell culture; Drs A. W. Ebeling (University of California, Santa Barbara) and H. E. Booke (Yale University) for reviewing and correcting the manuscript; and Dr F. H. Ruddle (Yale University) for the use of his facilities. This study was partially supported through U.S. Public Health Service Grant GM-09966 to Dr Ruddle.

REFERENCES

CHEN, T. R. MS, 1967. Comparative karyology of selected deep-sea and shallow-water teleost fishes. Ph.D. Dissertation. Yale University, New Haven, Conn.

CHEN, T. R., AND A. W. EBELING. 1966. Probable male heterogamety in the deep-sea fish *Bathylagus wesethi* (Teleostei: Bathylagidae). Chromosoma 18: 88–96.
1968. Karyological evidence of female heterogamety in the mosquitofish *Gambusia a. affinis* (Teleostei: Poeciliidae). Copeia 1968: 70–75.

CHEN, T. R., AND F. H. RUDDLE. 1970. A chromosome study of four species and a hybrid of the killifish genus *Fundulus* (Cyprinodontidae). Chromosoma. (In press.)

CHIARELLI, B., O. FERRANTELLI, AND C. CUCCHI. 1969. The caryotype of some teleostea fish obtained by tissue culture *in vitro*. Experientia 25: 426–427.

LEVAN, A., W. W. NICHOLS, M. PELUSE, AND L. L. CORIELL. 1966. The stemline chromosomes of three cell lines representing different vertebrate classes. Chromosoma 18: 343–358.

MCPHAIL, J. D., AND R. L. JONES. 1966. A simple technique for obtaining chromosomes from teleost fishes. J. Fish. Res. Bd. Canada 23: 767–768.

OHNO, S. 1965. Direct handling of germ cells, p. 75–90. *In* J. J. Yunis [ed.] Human chromosome methodology. Academic Press, Inc., New York.

REGAN, J. D., M. M. SIGEL, W. H. LEE, K. A. LLAMAS, AND A. R. BEASLEY. 1968. Chromosome alterations in marine fish cells *in vitro*. Can. J. Genet. Cytol. 10: 448–453.

ROBERTS, F. L. 1964. A chromosome study of twenty species of Centrarchidae. J. Morphol. 115: 401–418.

Morphological Differences in Lake Superior Lake Char

N. Y. KHAN AND S. U. QADRI

Department of Biology, University of Ottawa, Ottawa, Ont.

KHAN, N. Y., AND S. U. QADRI. 1970. Morphological differences in Lake Superior lake char. J. Fish. Res. Bd. Canada 27: 161–167.

Morphometric and meristic variations and ecological differences in 71 Lake Superior lake char, *Cristivomer namaycush*, from four localities confirmed that the lean lake char, *C. n. namaycush*, and the siscowet, *C. n. siscowet*, should be considered subspecies. They differed in four head characters and in mean numbers of anal fin rays and ribs. The siscowet and halfbreed differed in mean number of anal fin rays but not in any morphometric character studied. Differences between the humper and the three other forms were inconclusive.

Received October 29, 1968

IN THIS STUDY, an attempt was made to determine the extent of morphometric and meristic variations among the four forms of Lake Superior lake char, *Cristivomer namaycush*, and to assess their taxonomic status. In Lake

Printed in Canada (J1268)

Superior, several forms of lake char are recognized by fishermen and many fishery biologists by differences in spawning localities or dates, size at maturity, morphology, or colour. Four forms (the lean char, siscowet, halfbreed, and humper) are considered here. Lean char, the typical lake char, resembles those in the other Great Lakes. The siscowet and halfbreed are usually recognized by their excessive fat contents (Eschemeyer and Phillips, 1965; Thurston, 1962); the humper resembles the siscowet in general appearance but is distinguishable by thin ventral body walls and small size at maturity (Rahrer, 1965).

Some authors (Agassiz, 1850; Slastenenko, 1958) have considered the siscowet as a species, yet others (Jordan and Gilbert, 1883; Eddy and Surber, 1947) have considered it and lean lake char as subspecies. However, Hubbs and Lagler (1958) retained the subspecific ranking provisionally.

Materials and methods — A total of 71 char (Table 1), which came from four localities and were identified by fishermen as the lean lake char, siscowet, halfbreed, and humper, were considered for 15 morphometric and 7 meristic characters. On some specimens all characters could not be recorded. The humpers used were the same as those used by Rahrer (1965).

TABLE 1. Numbers of specimens of the four forms studied, capture data, and lengths.

Form	No. of specimens	Locality of capture	Depth of capture (*fath*)	Fork length (*mm*)	
				Range	Mean
Lean	15	1 mile W of MacInnes P., Ont.	6–20	422–515	437.8
Siscowet	19	Stannard Rock, 45 miles N of Marquette Mich.	65–70	331–775	557.2
Halfbreed	18	Point aux Mines Bank, Ont.	21–45	308–518	426.1
Humper	19	Isle Royale, 3–4 miles E of Rock Harbor	32–38	212–422	362.0

The morphometric measurements (Table 3) follow those of Hubbs and Lagler (1958) and Qadri (MS, 1964). The meristic characters (Fig. 1) were determined by radiographs and from specimens stained with alizarin red S. For the study of the morphometric characters the relative growth method developed by Huxley (1932) was used. All body measurements were regressed on fork length. The computations were done after logarithmic transformation of the data. The significance of regression coefficients was tested by a t-test at $P = 0.05$ and 0.01 (Steel and Torrie, 1960). For each meristic character, a one-way analysis of variance was performed and the significance of differences among the means was tested at $P = 0.05$ by the new Duncan's multiple range test with Kramer's modification (Steel and Torrie, 1960).

Morphometric characters — Most of the significant differences were observed in the head characters. The lean lake char had higher regression coefficients than the siscowet, halfbreed, and humper for all head characters except the diameter of the eye ball (Table 2, 3).

TABLE 2. Significance of differences in morphometric characters among the four forms.

Character	Lean vs. halfbreed		Lean vs. siscowet		Lean vs. humper		Halfbreed vs. siscowet		Halfbreed vs. humper		Siscowet vs. humper	
	df[a]	t[b]	df	t	df	t	df	t	df	t	df	t
Head length	29	4.896**	30	4.148**	30	3.428**	33	−0.161	33	−2.836**	34	−2.866**
Diam of eyeball	29	0.876	29	0.646	30	−1.129	32	−0.282	33	−4.107**	33	−4.093**
Postorbital distance	29	2.504*	30	3.018**	30	2.308*	33	0.718	33	−1.061	34	−2.239*
Interorbital width	29	2.767**	30	2.501*	30	2.847**	33	−1.604	33	−0.862	34	0.788
Length of upper jaw	29	4.896**	30	4.492**	30	1.891	33	−0.648	33	−1.246	34	−1.159
Length of lower jaw	29	4.559**	30	3.900**	30	3.033**	33	−0.419	33	−2.237*	34	−2.117*
Predorsal length	29	3.153**	30	1.653	29	2.796**	33	−1.072	32	−1.067	33	0.482
Ventral–anal distance	24	−1.451	25	−0.894	25	−0.941	33	0.788	33	1.387	34	0.494
Length of caudal peduncle	28	1.579	29	1.123	29	1.847	33	−0.772	33	0.088	34	0.982
Dorsal–adipose distance	19	1.695	20	1.070	20	2.024	33	−0.553	33	0.800	34	1.375
Depth of caudal peduncle	29	1.902	30	1.181	30	2.380*	33	−0.578	33	0.273	34	0.908
Pectoral–ventral distance	24	−0.350	25	−0.224	25	−0.294	33	0.602	33	0.338	34	−0.280
Height of dorsal fin	29	−0.028	30	−0.155	30	−0.391	33	−0.350	33	−0.885	34	−0.551
Height of anal fin	29	0.519	30	0.483	30	−0.544	33	−0.212	33	−1.914	34	2.012

[a]Degrees of freedom.

[b]Student's t-test values; * significant at $P = 0.05$; ** significant at $P = 0.01$.

TABLE 3. Regressions of lengths of head and body parts on fork lengths of four lake char forms from Lake Superior. Values in common logarithms.

Character	Form	Regression coefficient	Constant
Head length	Lean	1.475±0.105[a]	−1.889
	Siscowet	0.917±0.030	−0.419
	Halfbreed	0.908±0.040	−0.419
	Humper	1.071±0.039	−0.806
Diam of eyeball	Lean	0.613±0.165	−0.379
	Siscowet	0.476±0.472	−0.015
	Halfbreed	0.451±0.064	−0.035
	Humper	0.815±0.057	−0.897
Postorbital distance	Lean	1.481±0.168	−2.115
	Siscowet	0.964±0.033	−0.760
	Halfbreed	1.016±0.064	−0.919
	Humper	1.102±0.047	−1.108
Interorbital distance	Lean	1.698±0.225	−2.981
	Siscowet	0.154±0.040	−1.541
	Halfbreed	1.003±0.088	−1.161
	Humper	1.095±0.583	−1.385
Length of upper jaw	Lean	1.686±0.101	−2.726
	Siscowet	1.007±0.035	−0.940
	Halfbreed	0.960±0.060	−0.848
	Humper	1.139±0.121	−1.268
Length of lower jaw	Lean	1.618±0.126	−2.465
	Siscowet	0.997±0.035	−0.830
	Halfbreed	0.969±0.050	−0.782
	Humper	1.141±0.055	−1.196
Predorsal length	Lean	1.504±0.185	−1.649
	Siscowet	1.105±0.054	−0.591
	Halfbreed	1.011±0.035	−0.355
	Humper	1.065±0.034	−0.498
Ventral–anal distance	Lean	0.632±0.425	−0.254
	Siscowet	1.046±0.073	−0.840
	Halfbreed	1.148±0.078	−1.114
	Humper	0.984±0.083	−0.674
Length of caudal peduncle	Lean	1.286±0.158	−1.548
	Siscowet	1.015±0.056	−0.907
	Halfbreed	0.928±0.090	−0.693
	Humper	0.918±0.069	−0.649
Dorsal–adipose distance	Lean	2.223±0.522	−3.956
	Siscowet	1.166±0.080	−1.140
	Halfbreed	1.085±0.096	−0.920
	Humper	0.982±0.080	−0.659
Depth of caudal peduncle	Lean	1.425±0.170	−2.231
	Siscowet	1.099±0.065	−1.350
	Halfbreed	1.030±0.077	−1.210
	Humper	1.004±0.055	−1.102
Pectoral–ventral distance	Lean	0.976±0.682	−0.430
	Siscowet	1.098±0.048	−0.719
	Halfbreed	1.153±0.066	−0.871
	Humper	1.122±0.060	−0.803
Height of dorsal fin	Lean	0.899±0.245	−0.534
	Siscowet	0.939±0.050	−0.627
	Halfbreed	0.906±0.067	−0.510
	Humper	0.987±0.058	−0.715
Height of anal fin	Lean	1.059±0.139	−1.015
	Siscowet	0.976±0.037	−0.786
	Halfbreed	0.958±0.077	−0.729
	Humper	1.162±0.693	−1.247

[a]One standard error of coefficient.

Of the body measurements, the predorsal length was longer in the lean lake char than in halfbreed and humper. The lean lake char differed from the humper in these measurements in having a deeper caudal peduncle.

The humper had higher regression coefficients than the siscowet and halfbreed in head length, diameter of the eyeball, and length of lower jaw. The humper also had higher regression coefficients than the siscowet in postorbital distance.

The siscowet and halfbreed did not differ in any morphometric character.

The ventral–anal distance, the length of caudal peduncle, the dorsal–adipose distance, the pectoral–ventral distance, and the heights of dorals and anal fins were similar for all four forms.

Meristic characters — The mean number of branchiostegal rays was higher in the humper than in the siscowet, lean, and halfbreed (Fig. 1). The mean numbers of anal fin rays in the halfbreed and siscowet were different from each other and also from those for the humper and the lean lake char. The mean

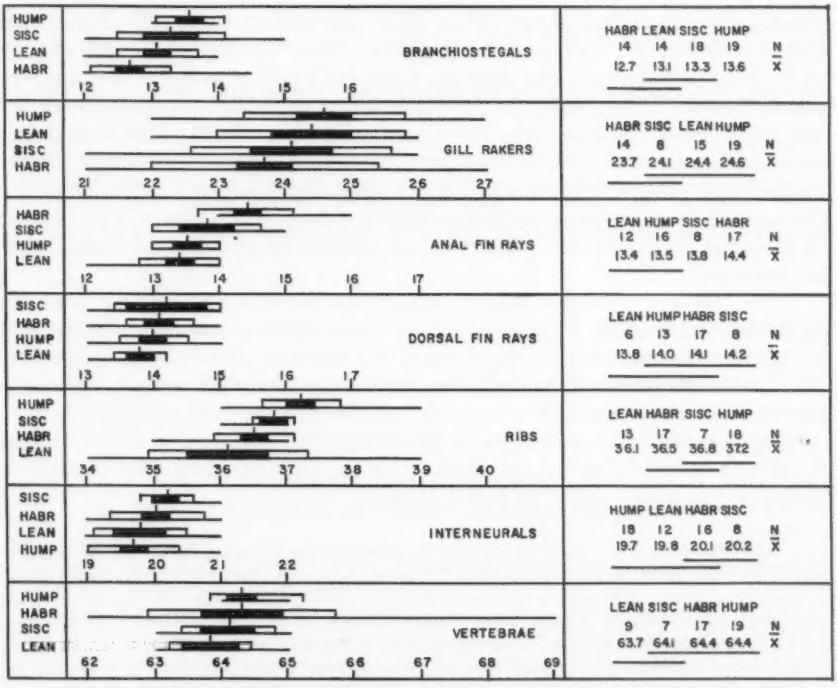

FIG. 1. Variation in meristic characters in the lean lake char, siscowet (SISC), halfbreed (HABR), and humper (HUMP) from Lake Superior. Horizontal lines, ranges; vertical lines on bars, means; solid bars, four standard errors of the means; and open and solid bars together, two standard deviations of the means. At right, any two means underscored by the same line are not different ($P = 0.05$) by Duncan's test.

numbers of ribs were lower in the lean lake char than in the other three forms. The siscowet and halfbreed showed similar values for all meristic characters except the branchiostegal rays and the anal fin rays.

The numbers of gill rakers, dorsal fin rays, interneurals, and vertebrae overlapped considerably in the four forms.

Discussion — On the basis of the morphological differences, the Lake Superior lake char forms three groups: (*a*) lean lake char, (*b*) siscowet and halfbreed, and (*c*) humper. It is still difficult, however, to judge the taxonomic status of these forms, except the lean lake char and the siscowet. The subspecific distinction between these two forms conforms with the two basic points in Mayer's (1965) definition of a subspecies, i.e., the separation of populations in space, and the taxonomic differentiation by diagnostic morphological characters.

The siscowet is separated from lean lake char spatially in terms of depth. The specimens studied were taken from deeper waters than the lean lake char (Table 1). However, the bathymetric distributions of these two forms overlap broadly (Eschmeyer, 1955, 1957). Recently, Crawford (MS, 1966) has worked out the average flotation pressure for the siscowet and lean lake char of Lake Superior. The average pressure was 6.3 atm for the siscowet and 3.8 atm for the lean lake char. From this Crawford concluded that the two forms would be neutrally buoyant at different depths. Morphologically most siscowets can be distinguished from the lean lake char in having relatively smaller and broader heads, and shorter jaws and opercles (Table 2, 3). On the basis of the morphological and ecological differences observed in this paper, we feel that the subspecific status of *C. n. namaycush* and *C. n. siscowet* should be retained.

The absence of morphological differences between the siscowet and the halfbreed suggests that they should be considered as one form. Eddy and Surber (1947) suspected that the siscowet and lean lake char hybridize in nature. The halfbreed could be the hybrids of the lean lake char and the siscowet; nevertheless they resemble siscowets morphologically and ecologically.

Since not much is known about the biology and distribution of the humper in relation to the other forms of lake char in Lake Superior, we hesitate to assign a taxonomic rank to the humper.

Acknowledgments — Cordial thanks are extended to the following for providing the lake char specimens: Mr A. H. Lawrie, Fisheries Research Board of Canada, Winnipeg, Man.; Dr D. E. McAllister, National Museums of Canada; Mr R. L. Pycha, U.S. Bureau of Commercial Fisheries, Ashland, Wisconsin; and Dr R. M. Bailey, Museum of Zoology, University of Michigan. Dr C. S. Shih of the Department of Agriculture of Canada, Ottawa, Ont., and Mr N. T. Gridgeman of the National Research Council, Ottawa, Ont., kindly offered their advice on the statistical treatment of the data. Dr S. I. Ahmad of the Electrical Engineering Department of the University of Ottawa wrote the computer programmes. The research was supported by grant A-2386 from the National Research Council of Canada.

REFERENCES

AGASSIZ, L. 1850. Lake Superior: its physical character, vegetation and animals, compared with those of other and similar regions. Gould, Kendall, and Lincoln, Boston, Massachusetts. 428 p.

CRAWFORD, R. H. MS, 1966. Buoyancy regulation in lake trout. M.A. Thesis. Univ. of Toronto, Toronto, Ont. 96 p.

EDDY, S., AND T. SURBER. 1947. Northern fishes with special reference to the upper Mississippi Valley. Revised ed. Univ. Minn. Press, Minneapolis, Minn. 276 p.

ESCHMEYER, P. H. 1955. The reproduction of lake trout in southern Lake Superior. Trans. Amer. Fish. Soc. 84: 47–74.

1957. The lake trout (*Salvelinus namaycush*). U.S. Fish Wildlife Serv. Fish. Leafl. 441: 11 p.

ESCHMEYER, P. H., AND A. M. PHILLIPS, JR. 1965. Fat content of the flesh of siscowets and lake trout from Lake Superior. Trans. Amer. Fish. Soc. 94: 62–74.

HUBBS, C. L., AND K. F. LAGLER. 1958. Fishes of the Great Lakes region. Revised ed. Cranbrook Inst. Sci. Bull. 26: 213 p.

HUXLEY, J. S. 1932. Problems of relative growth. Dial Press, New York. 276 p.

JORDAN, D. S., AND C. H. GILBERT. 1883. Synopsis of the fishes of North America. U.S. Nat. Mus. Bull. 12: 1–95.

MAYR, E. 1965. Animal species and evolution. The Belknap Press of Harvard Univ. Press, Cambridge, Mass. 797 p.

QADRI, S. U. MS, 1964. Osteology and morphology of the genera *Cristivomer* and *Salvelinus* and their relationships with other Salmonidae. Ph.D. Thesis. Univ. Ottawa, Ottawa, Ont. 296 p.

RAHRER, J. F. 1965. Age, growth, maturity, and fecundity of "humper" lake trout, Isle Royale, Lake Superior. Trans. Amer. Fish. Soc. 94: 75–83.

SLASTENENKO, E. P. 1958. The freshwater fishes of Canada. Kiev. Printers, Toronto, Ont. 385 p.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics with special reference to biological sciences. McGraw-Hill Book Inc., New York. 581 p.

THURSTON, C. E. 1962. Physical characteristics and chemical composition of two subspecies of lake trout. J. Fish. Res. Bd. Canada 19: 39–44.

Proportional Controlling of Water Temperatures

V. THIEL AND H. H. HARVEY

Department of Zoology
University of Toronto, Toronto, Ont.

THIEL, V., AND H. H. HARVEY. 1970. Proportional controlling of water temperatures. J. Fish. Res. Bd. Canada 27: 167–170.

In the regulation of water temperatures, it is now possible to improve on the conventional thermostat and the power switch it operates. This may be done through the use of a thermistor and a proportional controller. The latter supplies power to the heater in proportion to the difference between the temperature set on the dial and that measured by the thermistor. The result is exceptionally good temperature control with a minimum of mechanical problems.

Received September 8, 1969

Printed in Canada (J1585)

ABOVE AMBIENT, temperature control in tanks and aquaria is obtained most commonly by means of a thermostatically controlled on–off switch and an immersion heater. When the heater is on, it draws full power, resulting in rapid warming. When the heater is off, there is usually a period of gradual cooling. The result is a fluctuating water temperature. This occurs even when the demand temperature (the temperature called for on the thermostat) is very close to the actual temperature.

It is possible now to improve on both the thermostat and the on–off switch it operates. The thermostat may be replaced by a thermistor, and the switch by a proportional controller. A thermistor is a resistor that is highly dependent on temperature. Thermistors are available in a wide range of shapes and values. The resistive element is made from semi-conducting, oxidic materials. In this study, the thermistor used was a glass-enclosed thermometer-type (Philips E 205 CE/P2K2) cemented in a protective metal jacket. It has a resistance of approximately 1700 ohms at 35 C, 2200 ohms at 25 C, and 4700 ohms at 0 C. The change in resistance with variations in temperature controls the operation of the proportional controller.

The proportional controller consists of three major circuit sections: the Wheatstone bridge (with the set-point control potentiometer and the thermistor), a unijunction pulse circuit, and a TRIAC. For the purpose of this article it suffices to say that the bridge circuit produces an output voltage that depends on the settings of the control (temperature dial) and the temperature (and consequently the resistance) of the thermistor. This voltage is responsible for the rate of discharge in the unijunction circuit. Pulses on the output of the unijunction vary in phase with the half-cycles of the power frequency, occurring sooner or later depending on the voltage output from the off-balance condition of the bridge circuit.

TRIAC, the control element, is an AC semi-conductor switch, a development of the SCR (silicon-controlled rectifier). It is triggered into conduction by a gate signal from the unijunction circuit, on both half-cycles of the power line. The TRIAC is connected in series with the load and will conduct at various points in each half-cycle. For example, if the gate pulse triggers early in the cycle, full power is applied to the load, whereas if applied later in the cycle, the TRIAC switches into conduction for the remaining portion of the cycle, permitting less power being applied to the load (heater).

The thermistor and proportional controller act in a closed-loop, servo system where the feedback loop is completed from the heater through the aquarium to the thermistor (Fig. 1).

Proportional controllers are available (such as Model 70, RFL Industries, Boonton, N.J.) that contain all of the circuitry, and with only five external wires to be connected (Fig. 2). Model 70 will control heaters up to a capacity of 1500 w, with a suitable thermistor between 100 and 8000 ohms nominal value. In this study, the above unit was custom-fitted to its own case, complete with input cord, output socket, and indicator lamp. The controller was coupled with a thermistor of 2.2 k ohms nominal resistance and a 800-w heater in an

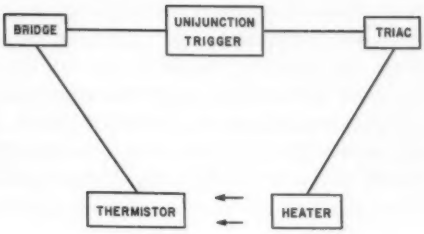

FIG. 1. Block diagram of the proportional-controller feedback circuits.

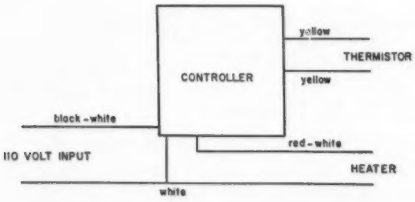

FIG. 2. Connection diagram of the Model 70 RFL proportional controller.

aquarium of 20 U.S. gal capacity. Aeration provided the required mixing and movement of water past the thermistor. The power that the controller supplied to the heater was measured for constructing Fig. 3. At a demand temperature of 0.5 C above ambient, the controller supplied 25 w, at 1.0 C it supplied 300 w, at 2.0 C 725 w, etc. Thus for any setting above ambient temperature, the controller supplied power in the amount shown, and with power diminishing as the aquarium temperature approached demand temperature. Within a

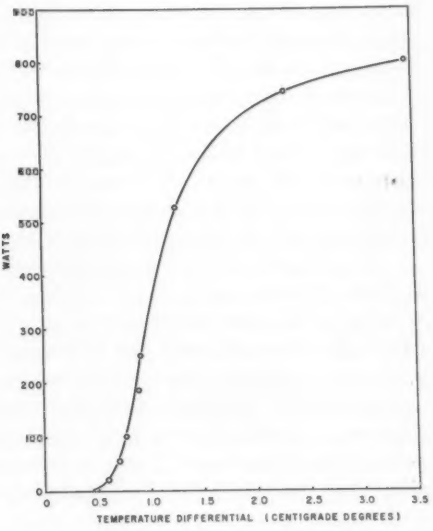

FIG. 3. Power applied to heater in response to temperature differential between aquarium (thermistor) and demand (dial) temperature.

short time after any new temperature was called for (by setting the potentiometer) the controller was supplying power at a rate that balanced heat lost from the system, resulting in an exceptionally stable water temperature.

The advantages of the proportional controller are better control, longer life (no moving parts, contacts, tubes, or relays), and less fragility. Cost of the controller ($40.00) is approximately that of a good quality, mercury-switch thermostat. Suitable thermistors are available for less than a dollar.

First Specimens of the Stonecat, *Noturus flavus*, from the Hudson Bay Drainage

K. W. Stewart and C. C. Lindsey

Department of Zoology
The University of Manitoba, Winnipeg 19, Man.

Stewart, K. W., and C. C. Lindsey. 1970. First specimens of the stonecat, *Noturus flavus*, from the Hudson Bay drainage. J. Fish. Res. Bd. Canada 27: 170–172.

Nine specimens of stonecat were taken in the Red River 16 km south of Winnipeg, Man. This is the only known collection of the species from the Hudson Bay drainage. Ecological factors seem to be the main influence confining the species to its present restricted northern locality.

Received September 24, 1969

Nine specimens of the stonecat, *Noturus flavus* Rafinesque, including three maturing females, were caught in the Red River about 16 km south of the center of Winnipeg, Man., between June 29 and August 4, 1969. All but two of the specimens were captured by K. W. Stewart angling with a worm bait near midstream, about 100 m downstream from the control gates of the Red River Floodway (about 300 m south of the mouth of La Salle River). The remaining specimens were caught by J. G. Eales, angling in the same locality. The river was moderately fast, turbid, and had a mud bottom, with a depth of about 3 m and a width of about 150 m.

On June 30, 1969 the authors made another collection in the area, using setlines, gillnets, and minnow traps. Setlines and gillnets were also used to sample in the area during August 1967. None of these collections yielded *N. flavus*, but they included the following species (listed in descending order of frequency): sauger (*Stizostedion canadense*), black bullhead (*Ictalurus melas*), channel catfish (*Ictalurus punctatus*), burbot (*Lota lota*), walleye (*Stizostedion vitreum*), tadpole madtom (*Noturus gyrinus*), emerald shiner (*Notropis atherinoides*), spottail shiner (*Notropis hudsonius*), carp (*Cyprinus carpio*), northern pike (*Esox lucius*), brown bullhead (*Ictalurus nebulosus*), and freshwater drum

Printed in Canada (J1596)

(*Aplodinotus grunniens*). Only one specimen was taken of each of the last four species. All five species of catfish known from Manitoba are present in this area.

The first specimen of *N. flavus* captured is deposited in the National Museum of Natural Sciences (Cat. No. NMC 69-107); the remaining specimens are in the Department of Zoology, University of Manitoba. Counts and measurements (following the methods of Hubbs and Lagler, 1967) of three of the specimens are as follows (in each instance the first entry being the first specimen, NMC 69-107):

Standard length 113.7 mm, 130.9 mm, 135.0 mm; dorsal II-6, II-6, II-6; anal 17, 15, 16; left pectoral I-11, I-10, I-10; right pectoral I-11, I-10, I-10; left pelvic 9, 9, 9; right pelvic 9, 9, 9; vertebrae 45 (last 2 centra partly fused), 44, 44. These specimens, the most northerly ever reported, have high vertebral counts compared with the range (41–44) recorded by Cross (1967).

In Alberta, *N. flavus* is probably confined to Milk River, tributary to the Missouri River (Nursall and Lewin, 1964). The species has not been recorded from Saskatchewan. It occurs in southern Minnesota (Eddy and Surber, 1947), the southern two-thirds of Wisconsin and Michigan, southern Ontario, and the upper St. Lawrence River; its southern limit is the Tennessee River system of northern Alabama (Hubbs and Lagler, 1967).

This is the first authenticated record of *N. flavus* from the Red River drainage, and from Manitoba. Bissett (1927) listed the species as occurring in Manitoba, but gave no indication of locality. Scott (1958) concluded that the listing by Bissett was probably an error. Bailey and Allum (1962) indicated that *N. flavus* "apparently reached the Big Stone Lake area [the headwaters of the Minnesota River basin] too late to utilize the Warren River waterway into the Red River." Underhill (1957) stated that in Minnesota the species is absent from the Red River, upper Mississippi River, and Lake Superior basins. Fedoruk (1969) listed the species under "Unacceptable Occurrences" in his checklist of Manitoba fishes. No specimens were present in any previous collections made in the Red River in Manitoba by the senior author.

The habitat in the locality where the specimens were taken has been altered by man within the past decade. Water velocity has been increased by the constriction of the channel by the floodway gates, and limestone boulder fill extends offshore a short distance from both river banks. Habitat alteration caused by the floodway has perhaps created a suitable environment for *N. flavus* where none existed before. This may have allowed the survival of stonecats for the first time, or, more likely, could have caused a concentration of what is otherwise a rare and scattered species in the Red River. Whether their invasion from the south was recent or ancient, the species probably entered from the Minnesota River (a tributary of the Mississippi). Stonecats may have moved northward into glacial Lake Agassiz while that lake was discharging south by the Warren River outlet (now occupied by the northward flowing Red River). Alternatively, they may have moved northward over the divide more recently. Even in modern times there are temporary connections during

spring floods between the Minnesota River and Red River at Lake Traverse (Underhill, 1957).

REFERENCES

BAILEY, R. M., AND M. O. ALLUM. 1962. Fishes of South Dakota. Misc. Publ. Mus. Zool. Univ. Mich. 119: 131 p.

BISSETT, E. D. R. 1927. Fresh-water fish of Manitoba. Can. Field Natur. 41: 127–128.

CROSS, F. B. 1967. Handbook of fishes of Kansas. Univ. Kans. Mus. Natur. Hist. Misc. Publ. 45: 357 p.

EDDY, S., AND T. SURBER. 1947. Northern fishes, with special reference to the Upper Mississippi Valley. Rev. ed. Univ. Minnesota Press, Minneapolis, Minn. 276 p.

FEDORUK, A. N. 1969. Checklist of and key to the freshwater fishes of Manitoba (Preliminary). Manitoba Dep. Mines Natur. Resour., Can. Land Inventory Proj. Rep. 6: 98 p.

HUBBS, C. L., AND K. F. LAGLER. 1967. Fishes of the Great Lakes region (with a new preface). Univ. Michigan Press, Ann Arbor, Mich. 213 p.

NURSALL, J. R., AND V. LEWIN. 1964. The stonecat, *Noturus flavus*, newly recorded in Alberta. Can. Field Natur. 78: 128–129.

SCOTT, W. B. 1958. A checklist of the freshwater fishes of Canada and Alaska. Roy. Ont. Mus., Div. Zool. Palaeontol., Toronto, Ont. 30 p.

UNDERHILL, J. C. 1957. The distribution of Minnesota minnows and darters in relation to Pleistocene glaciation. Occas. Pap. Minn. Mus. Natur. Hist. 7: 45 p.

Exact Statistical Inferences about the Parameter for an Exponential Growth Curve following a Poisson Distribution

J. K. LINDSEY[1]

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

LINDSEY, J. K. 1970. Exact statistical inferences about the parameter for an exponential growth curve following a Poisson distribution. J. Fish. Res. Bd. Canada 27: 172–174.

A use of the relative likelihood function is described. An example is provided to show exact statistical inferences about the parameter for an exponential increase in cell concentration when the concentration is a Poisson random variable.

Received August 22, 1969

SUPPOSE that the concentration of some organism increases exponentially from some known starting level P_0 at time $t = 0$, to a level P_t at time t. Then,

---

[1]Present address: Department of Mathematics, Imperial College, University of London, London, England.

Printed in Canada (J1573)

$$P_t = P_0 e^{kt} + \text{error.}$$

If a small sample measuring concentration is removed from a larger culture, it may be assumed to have the Poisson distribution. Then the mean value of P_t is

$$E(P_t) = P_0 e^{kt} = \mu \tag{1}$$

and the distribution of P_t is

$$f(P_t; k) = \frac{e^{-\mu} \mu^{P_t}}{P_t!} = \frac{e^{-P_0 e^{kt}} P_0^{P_t} e^{ktP_t}}{P_t!}. \tag{2}$$

After a value of P_t has been observed, the likelihood function (see Sprott and Kalbfleisch, 1965; for more details see Barnard et al., 1962, and Sprott and Kalbfleisch, 1969) for k is proportional to

$$L(k) = \exp\{ktP_t - P_0 e^{kt}\}. \tag{3}$$

This function is maximized at the maximum likelihood estimate,

$$\hat{k} = \frac{\log P_t - \log P_0}{t}. \tag{4}$$

The precision of this estimate is given by the relative likelihood function

$$R(k) = \frac{\exp\{ktP_t - P_0 e^{kt}\}}{\exp\{\hat{k}tP_t - P_0 e^{\hat{k}t}\}} \tag{5}$$

which may be plotted for various values of k ($0 \leq R \leq 1$). Values of $R > 0.1$ might be considered to give a reasonable interval for k, comparable with a 95% probability interval for a normal theory linear model.

The use of the relative likelihood avoids the customary normal theory approximation; inferences using relative likelihoods are exact and in addition, in this example, require fewer calculations than the approximate methods. More complicated growth curves (e.g., von Bertalanffy curve) are amenable to the same analysis and are essentially as easy to deal with due to present day accessibility of high speed computers.

Suppose that the initial concentration of cells is measured as 20 cells per milliliter and that after 5 hr this has increased to 100 cells per milliliter. Then, $P_0 = 20$, $P_5 = 100$, $t = 5$

$$R(k) = \exp\{500k - 20e^{5k} - 60.944\}$$
$$\hat{k} = 0.32189$$

from equations 5 and 4. Values of the relative likelihood function are:

k	0.1	0.2	0.28	0.3	0.32189	0.36	0.37	0.38	0.4
R	10^{-19}	10^{-7}	0.117	0.475	1.0	0.143	0.0432	0.0237	10^{-3}

Then a 10% relative likelihood interval (Fig. 1) is about (0.278, 0.362).

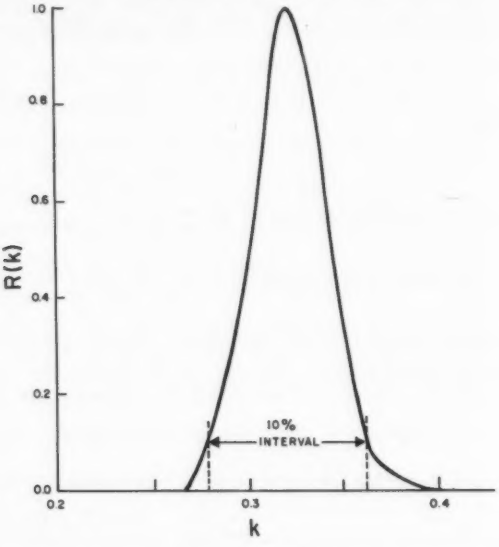

FIG. 1. Relative likelihood function for k.

Acknowledgments — The author would like to express his appreciation to C. D. McAllister for bringing this problem to his attention.

REFERENCES

BARNARD, G. A., G. M JENKINS, AND C. B. WINSTEN. 1962. Likelihood inference and time series. J. Roy. Statist. Soc. Ser. A 125: 321–372.

SPROTT, D. A., AND J. D. KALBFLEISCH. 1969. Examples of likelihoods and comparison with point estimates and large sample approximations. J. Amer. Statist. Ass. 64: 468–484.

SPROTT, D. A. AND J. G. KALBFLEISCH. 1965. Use of the likelihood function in inference. Psychol. Bull. 64: 15–22.

The Manefish, *Caristius groenlandicus* Jensen (Percomorphi: Caristiidae), in Atlantic Waters off Canada

W. B. SCOTT,[1] A. C. KOHLER,[2] AND R. E. ZURBRIGG[3]

SCOTT, W. B., A. C. KOHLER, AND R. E. ZURBRIGG. 1970. The manefish, *Caristius groenlandicus* Jensen (Percomorphi: Caristiidae), in Atlantic waters off Canada. J. Fish. Res. Bd. Canada 27: 174–179.

Morphometric and meristic data for the first recorded captures of *Caristius groenlandicus* in Canadian Atlantic waters in 1966 (1) and 1968 (7) are described and compared with previously published records.

Received July 25, 1969

[1]Royal Ontario Museum and University of Toronto, Toronto, Ont.
[2]Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B.
[3]Department of Zoology, University of Toronto, Toronto, Ont.

Printed in Canada (J1541)

ON THE AFTERNOON OF APRIL 20, 1966, a specimen of the manefish, *Caristius groenlandicus*, was caught in a #41 Yankee trawl, along with haddock, *Melanogrammus aeglefinus*, in 146.3 m on the northeast peak of Georges Bank by the otter trawler *Verna and Gean*.

This fish was recognized as unusual and was sent to the Fisheries Research Board of Canada Biological Station at St. Andrews, N.B. There the specimen was identified by the senior author as a manefish but because of certain apparently unusual features publication was withheld pending further investigation. Correspondence revealed that additional specimens had been captured southward of the present record (Dr G. W. Mead, personal communication) and the confusion regarding the taxonomy of the Caristiidae also became obvious.

In July 1968, the M/V *Brandal*, on charter to the Fisheries Research Board of Canada Marine Ecology Laboratory at Dartmouth, N.S., was engaged in a survey of the mesopelagic fauna in specific regions of the Grand Bank and northward, using a specially constructed midwater trawl. The junior author participated in this cruise at the invitation of the Fisheries Research Board, because of his studies of the lanternfishes (Myctophidae). During the cruise seven more specimens of the manefish were captured, between the depths of 0–1100 m. This trawl required ½ hr to reach fishing depth and a further hour to haul in. Time spent at the fishing depth (1100 m) ranged from 1 to 3 hr. It is impossible to delineate the actual depth of capture, since the net did not have a closing device.

A single previous report of a manefish in the western North Atlantic was published by Jensen (1941), who reported a specimen caught on June 9, 1909, in Davis Strait, off the coast of Greenland. Jensen considered his specimen to differ sufficiently from previously described forms to warrant designating it as a new species, *Caristius groenlandicus*.

Morphometric data for all specimens mentioned (including Jensen's) are presented in Table 1. Measurements and counts were made as described by Hubbs and Lagler (1958), including the method of counting caudal fin rays as the number of branched rays, plus two. The specimen caught in 1966 (Fig. 1) was not severely damaged, although the enlarged dorsal and pelvic fins were broken and frayed. The 1968 specimens are in comparable condition.

A description of the specimens follows. The teeth are uniserial on the jaws, vomer, and palatines. Pseudobranchae are present. On all specimens the body and head are covered with thin cycloid scales having widely spaced circuli. The scale illustrated by Jensen is similar in shape and circuli pattern to the scales examined from our specimens. The scales are rather irregularly arranged on the body, as noted by Norman (1930) for *Caristius macropus* (Bellotti). Scales also occur on the inside of the extensive ventral groove, into which the enlarged pelvic fins can be folded. The base of the dorsal fin is also enclosed in a groove, although no scales were observed on the inner walls. It is of special interest to note Jensen's remarks (p. 50) concerning the presence of dorsal and ventral grooves: "The basal part of the anal fin is, like the dorsal fin, covered by a sheath of skin, the two leaves of which diverge at the front end of the anal fin, forming between them a fairly broad longitudinal groove, extending right to the place of attachment of the ventral fins; when the ventral fins are laid flat, they can be enclosed in this groove." The lateral line is present and is arched. The dorsal and anal fin ray counts are given as a single figure, without

TABLE 1. Morphometric and locality data for nine specimens of *Caristius groenlandicus*.

Morphometric and meristic values[a]													ROM[b] Cat. No.	Date of capture	Position and depth of capture
							Rays								
T.L.	S.L.	Hd.L.	Sn.L.	Eye	B.D.	C.P.D.	D	A	Pect.	Pelv.	C	Vert.			
187.6	154.3	44.0	5.0	20.4	74.0	22.5	33	20	17	I,5	17	36	26553	July 18, 1968	46°N; 44°30′W 0–1000 m
190.4	165.0	45.9	6.4	19.0	77.8	23.7	33	20	17	I,5	17	37	26552	July 12, 1968	50°30′N; 49°30′W 0–1000 m
198.0	170.0	42.5	6.0	20.0	80.0	19.0	33	20	17	I,5	17	35	26008	Apr. 20, 1966	45°50′N; 65°46′W 146.3 m
203.6	170.0	44.5	5.8	17.0	80.6	21.9	34	21	16	I,5	17	36	26582	July 24, 1968	42°30′N; 50°W 0–1100 m
227.0	192.4	51.9	6.0	19.0	91.4	28.7	32	21	17	I,5	17	35	26555	July 24, 1968	42°30′N; 50°W 0–1100 m
228.0	197.0	49.9	7.5	18.0	95.0	29.0	33	19	17	I,5	17	36	26556	July 19, 1968	45°30′N; 47°30′W 0–1025 m
240.4	207.0	52.0	7.1	20.5	95.0	28.9	32	19	17	I,5	17	36	26554	July 19, 1968	45°30′N; 47°30′W 0–1025 m
253.6	213.0	53.0	7.0	20.0	90.0	29.0	32	18	17	I,5	17	35	26557	July 17, 1968	47°30′N; 43°W 0–1000 m
172.0		37.0		15.0			34	21	17	I,5	19		Jensen (1941)	June 9, 1909	62°53′N; 54°15′W "(1500 & 1200 M.W.)"

[a]T.L. = total length; S.L. = standard length; Hd.L. = head length; Sn.L. = snout length; Eye = eye diameter; B.D. = body depth; C.P.D. = caudal peduncle depth; D = dorsal fin rays; A = anal fin rays; C = caudal rays; Pect. = pectoral rays; Pelv. = pelvic rays; Vert. = number of vertebrae.
[b]ROM = Royal Ontario Museum.

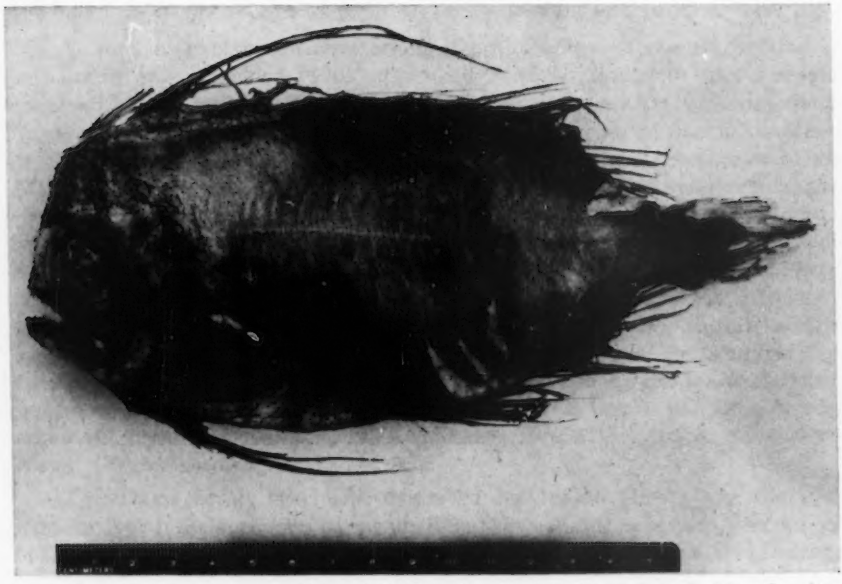

FIG. 1. *Caristius groenlandicus*, ROM Cat. No. 26008, 170.0 mm standard length.

Scott et al. — J. Fish. Res. Bd. Canada

differentiating spines and rays, except for the spine preceding the 19 or 20 anal rays, which is quite short (2 or 3 mm). Both Jensen (1941) and Maul (1949) (for *Platyberyx opalescens*) remark on the tiny spines on the ventral rays of the caudal fin. The vertebral counts were made from X-ray plates and include all fully formed centra but do not include the urostyle. The vertebral counts for the eight specimens were 35(3), 36(4), and 37(1). The vertebrae, especially the anterior ones, were difficult to count and, therefore, a dissection was made on specimen no. 26552 to verify the counts made from X-ray plates. The only previous counts known to us were given as "...about 40..." by Gill and Smith (1905) for *Caristius japonicus* from the Pacific basin, near Japan, and 37 and 39 for *P. opalescens* Zugmayer by Koefoed (1953). Note that all of our specimens have 17 caudal rays (15 branched), and a pelvic fin ray formula of one spine and five branched rays. All specimens have seven branchiostegals, four on the ceratohyal and three on the epihyal. Colour of freshly caught specimens was pinkish-brown body with black fins; the transluscent flesh of the body wall permits the dark peritoneum to show through, especially ventrally; iris of the eye is golden.

A word of explanation concerning the taxonomy of the Caristiidae is perhaps in order. Bellotti (1903) described *Pteraclis macropus* from a single specimen, 110 mm long, taken off Japan. Gill and Smith (1905) described *C. japonicus* also from a single specimen (75 mm long) found in a fish market in Yokohama, Japan. Zugmayer (1911) provided the first description of an Atlantic form and described as *Platyberyx opalescens* a 65-mm specimen caught south of Cape St. Vincent, Portugal. Regan (1912) considered *P. opalescens* to be congeneric with *C. japonicus*, perhaps conspecific. He also agreed with Zugmayer's decision that the species belonged in the Berycomorphi, largely because of the 17 branched caudal rays. Jordan (1919), in a review of related species, created a new genus, *Elephenor*, and family, Elephenoridae, for *Pteraclis macropus* Bellotti, but Norman (1930) placed *Elephenor* in the synonymy of *Caristius*, where it has remained. Subsequently, two single south Atlantic specimens were referred to the species *C. macropus* (Bellotti): one, 60 mm long taken off Argentina (Norman, 1930), and one, 30 mm long, taken off western Africa (Fraser-Brunner, 1931).

The Davis Strait specimen, described by Jensen (1941) was the first western North Atlantic record, again based on a single specimen, but larger (172 mm) than those previously reported. Jensen chose to record his specimen as a new species, *C. groenlandicus*. He also provided a review of the previous records of caristiid fishes because, as he stated "authors are undecided in the determination of the few specimens hitherto known." The caudal fin ray count, when reported for any of the previously caught specimens, was given as 17, except for Koefoed, who reported 15. The discrepancy in caudal fin ray counts may have resulted from a difference in the method of enumeration.

Maul (1949) described *C. maderensis* as new, an eastern Atlantic form found off Madeira and West Africa. The description was based on two specimens, both females. Judging from the illustration, *C. maderensis* is a small-eyed form in which the maxillary reaches only to the anterior margin of the eye, and the pelvic fins appear to originate in advance of the dorsal origin. Maul also noted, for one specimen, that the pelvics cannot be totally accommodated within the ventral groove. He states (p. 23): "Anal and posterior half of dorsal

deeply sunk into body. There is a deep groove between ventral and anus. The ventrals are somewhat longer than this groove, so that, when retracted, they cannot be received entirely within this groove, their soft flabby ends appear beyond the anus. The lower half of the scaly fleshy covering of the anal is loose, but there is no connection between the ventral groove and the two loose parts on either side of the anal, which would form a biforked continuation of the ventral groove, so that the ventrals cannot be hidden in their entire length." The pelvics could be accommodated in the ventral groove of the second specimen. In the same paper, Maul also described a specimen (115 mm) of *Platyberyx opalescens* Zugmayer, which more closely resembles our specimens in fin ray counts but which differs markedly by having a smaller eye, among other features. The caudal fin ray count was not given for either species.

Koefoed (1953), reporting on Michael Sars North Atlantic collections, recorded two small specimens, 35 and 63 mm long, as *P. opalescens* Zugmayer. Koefoed noted that his specimens had 15 branched caudal rays. Wheeler (1968) also reported *C. macropus* from Icelandic waters and southwest of Ireland but no details were given.

The inconsistencies and general vagueness of morphometric values published in the literature suggest that the Caristiidae and related forms are in need of serious study and revision. Also, it seems certain that more than one species has been included in *C. macropus* (Bellotti). Indeed, the same may be true for *C. japonicus* and *P. opalescens*.

Caristius japonicus (Gill and Smith) and *C. groenlandicus* Jensen appear to be large-eyed forms, with the pelvics distinctly in advance of the pectorals, whereas *C. macropus* (Bellotti) and *P. opalescens* Zugmayer have smaller eyes and the pelvic fins are located more or less below the pectorals. Since our specimens are obviously the large-eyed form with advanced pelvics, they belong to the *C. japonicus* group, but since *C. japonicus* and *C. groenlandicus* may be different species, we prefer to use the name provided by Jensen (1941), *C. groenlandicus*, on geographic grounds. Jensen's specimen agrees closely with ours in most morphometric and meristic values, except in the number of caudal rays, for which ours are consistently 15 branched.

The presence (usually) of 15 branched caudal rays and I,5 pelvic fin ray formula suggests that the family belongs in the Perciformes, as indicated by Greenwood et al. (1966), although most authors have referred the family to the Beryciformes (Regan, 1912; Norman, 1930; Koefoed, 1953; McAllister, 1968).

Although we do not know with certainty the depth at capture of these specimens, it is noteworthy that all were captured at the continental shelf margin, on or about the 2000 m line. Jensen's specimen also would seem to have been taken on the shelf margin. It is perhaps significant that our eight specimens are all females, and both specimens of *C. maderensis* described by Maul were females. Sex was not reported for other specimens noted in the literature, but many of these were of small size and probably immature.

It is our hope that the observations noted above will be useful to whomever undertakes the sorely needed worldwide revision of this group of fishes.

Acknowledgments — We are grateful to Captain M. McKenzie, Jr., of the *Verna and Gean*, and Fisheries Research Board port technician D. Lyon for recognizing the importance of the 1966 specimen and retaining it for study. We are also grateful to Dr L. M. Dickie and Mr E. Bakken, Fisheries Research Board of Canada, Marine Ecology Laboratory, Dartmouth, N.S., for access to the 1968 specimens. The 1966 specimen was photographed by Mr W. McMullon. All specimens are stored in the Royal Ontario Museum research collections. This study was made possible, in part, by a National Research Council grant, No. A4284, to the senior author.

REFERENCES

BELLOTTI, C. 1903. Di un nuovo pteraclide Giapponese. Atti. Soc. Ital. Sci. Natur. Mus. Civico Storia Natur. Milano 42: 136–139.

FRASER-BRUNNER, A. 1931. Some interesting West African fishes, with description of a new genus and two new species. Ann. Mag. Natur. Hist. 10: 217–225.

GILL, T., AND H. M. SMITH. 1905. A new family of jugular Acanthopterygians. Proc. Biol. Soc. Wash. 18: 249–250.

GREENWOOD, P. H., D. E. ROSEN, S. H. WEITZMAN, AND G. S. MYERS. 1966. Phyletic studies of teleostean fishes, with a provisional classification of living forms. Bull. Amer. Mus. Natur. Hist. 131: 339–456.

HUBBS, C. L., AND K. F. LAGLER. 1958. Fishes of the Great Lakes region. Cranbrook Inst. Sci. Bull. 26: 1–186.

JENSEN, Ad. S. 1941. A new deep sea berycoid fish from Davis Strait *Caristius groenlandicus* n.sp. Vidensk. Medd. Naturhist. Foren. Bd. 105: 49–53.

JORDAN, D. S. 1919. On *Elephenor*, a new genus of fishes from Japan. Ann. Carnegie Mus. 12: 329–334.

KOEFOED, E. 1953. Synentognathi, Solenichthyes, Acacanthini, Berycomorphi, Xenoberyces from the 'Michael Sars' North Atlantic deep sea expedition 1910. Rep. Sci. Results. Michael Sars N. Atl. Deep Sea Exped. 1910 4(pt. 2): 38 p.

MAUL, G. E. 1949. Alguns peixes notáveis. Bol. Museu Munic. Funchal 4: 22–30.

MCALLISTER, D. E. 1968. Evolution of branchiostegals and classification of teleostome fishes. Nat. Mus. Can. Bull. 221: 239 p.

NORMAN, J. R. 1930. Oceanic fishes and flatfishes collected in 1925–27. Discovery Rep. 2: 261–370.

REGAN, C. T. 1912. The Caristiidae, a family of berycomorphous fishes. Ann. Mag. Natur. Hist. 8: 637–638.

WHEELER, A. 1968. The fishes of the British Isles and north-west Europe. Macmillan & Co. Ltd., Toronto, Ont. 613 p.

ZUGMAYER, E. 1911. Diagnoses des poissons nouveaux provenant des campagnes du yacht "Princess-Alice" (1901 à 1910). Bull. Inst. Oceanogr. (Monaco) 193: 14 p.

Estimates of Seston Crops by Filtration with Glass Fiber Discs

DALE F. REED

Colorado Game, Fish and Parks Division
Glenwood Springs, Colorado, USA

AND

EDWARD B. REED

Department of Zoology
Colorado State University, Fort Collins, Colorado 80521, USA

REED, D. F., AND E. B. REED. 1970. Estimates of seston crops by filtration with glass fiber discs. J. Fish. Res. Bd. Canada 27: 180–185.

Glass fiber filters were compared with centrifugation as a means of concentrating seston from a small fluctuating reservoir. Fifteen sets of samples drawn over a 2-month period showed that the filters removed about twice as much seston, by dry weight, as did the centrifuge.

Received September 2, 1969

THE CONTINUOUS FLOW CENTRIFUGE has long been used to extract seston, particularly the nannofraction, from lake water. Plankton investigators have presented a variety of solutions to the problem of loss of low-density particles during centrifugation. Pennak (1949) applied a correction factor of 25%; Hartman (1958) recommended passing water through the centrifuge six times to affect complete removal of seston. Kerekes (1966) centrifuged samples three times and added 11% to correct for incomplete removal.

An additional problem is that large amounts of water from oligotrophic lakes must be centrifuged to secure suitable catches for reliable weighing.

Preliminary use of glass fiber filters suggested that small amounts of water, even from oligotrophic mountain lakes, were sufficient for reliable gravimetric determinations of seston. Therefore, the primary objective of this study was to compare the effectiveness of glass fiber filters with centrifugation in removing seston from natural lake water containing mixed populations of phyto- and zoo-plankters as well as organic and inorganic detritus.

In view of current interest in the extraction of pigments from seston, a secondary objective was to compare optical densities of extracts from seston obtained by filtration with those obtained by centrifugation. Corcoran et al. (1964) demonstrated that glass fiber filters do not adversely affect absorption spectra.

Printed in Canada (J1582)

Methods — Dixon Reservoir, a small 4-ha irrigation reservoir near Fort Collins, was chosen for sampling. All samples were taken from the vertical headgate structure, where the depth ranged up to 4 m. A 3-liter Kemmerer bottle, .5 m in length, was positioned so that six samples included a column extending from the surface to 3 m. The upper three samples were mixed in the field by emptying the bottle into a large plastic container. The lower three samples were composited in a second container. Samples were returned without preservation to the laboratory where analyses began within an hour of collection. Samples were drawn on 15 days between June 1 and July 29, 1968.

In the laboratory, the water in each container was thoroughly mixed and subsampled in duplicate according to the following procedures.

(1) 100 ml of sample were drawn through a fiber-glass filter (Whatman Grade GFC 4.25-cm ultra-fine filter with semi-colloidal range) by vacuum pump. The fiber-glass filter and seston were submerged in 10 ml of 90% aqueous acetone while the filter was still wet and placed in a dark refrigerator for 12–24 hr. Another 100 ml of sample were drawn through another, pre-weighed, filter. The filter was then dried 12–24 hr at 100 C and weighed to determine dry weight and then placed in 10 ml of acetone and extracted in a refrigerator for 12–24 hr. Extracts were passed through filter paper saturated with 90% acetone and adjusted to a constant volume. Optical density was determined with a B & L Spectronic 20 set at 665 nm.

Thus from the fiber-glass method three variables were sampled in duplicate: optical density of wet filter, optical density of dry filter, and dry weight.

(2) One liter of sample was centrifuged in a Foerst Electric centrifuge with a maximum speed of 20,000 rpm. Flow was adjusted so that 6–7 min were required for 1 liter to pass through the instrument. This is the flow rate suggested in Standard Methods (American Public Health Association, 1965); Welch (1948) stated that 7–10 min are required. The catch was carefully scraped into a tared crucible and dried at 100 C for 12–24 hr, cooled in a desiccator, and weighed.

Results — Mean dry weight of filter catches was about twice that of centrifuge catches in the upper depth, and not quite double at the lower. Thus it seems clear that the filters retained more seston than the centrifuge (Table 1). The mean optical density of dry filters was about half that of the wet filters. Differences in filter samples suggest that drying in some way rendered pigments or other extractable materials less soluble. In view of the admonition by Strickland and Parsons (1960) to extract pigments in the dark at cool temperatures, this is not surprising.

TABLE 1. Fiber-glass filtration vs. centrifugation estimates of standing crop of seston, Dixon Reservoir.

Estimator	Range	Mean	Variance	SE	95% confidence limits
Upper depth					
Optical density, wet filter	.123–1.029	.5115	.09903	.08125	.3372–.6858
Optical density, dry filter	.039–.605	.2085	.02172	.03938	.1229–.2401
Dry wt, filter, *mg/liter*	10.5–41.8	29.0267	138.22924	3.03567	22.515–35.5382
Dry wt, centrifuge, *mg/liter*	7.6–20.9	14.5533	16.44981	1.04721	14.5055–23.5345
Lower depth					
Optical density, wet filter	.114–.841	.4453	.05938	.06292	.3103–.5803
Optical density, dry filter	.083–.414	.1815	.01031	.02714	.1229–.2401
Dry wt, filter, *mg/liter*	10.0–55.5	33.4467	197.68552	3.63028	25.6597–41.2337
Dry wt, centrifuge, *mg/liter*	7.5–35.6	19.0200	66.44314	2.10465	14.5055–23.5345

Dry weight determined by centrifugation was less variable than filter dry weight (Table 2), but was significantly smaller by paired *t*-test (Table 3). Optical density obtained from wet filters was significantly greater than that from dried filters. Overlaps of confidence intervals (Table 1) indicate that mean crops in the upper stratum did not differ significantly from those in the lower.

TABLE 2. Coefficients of variability of estimates of seston crops.

Estimator	Depth	Coefficient of variability
Optical density, wet filter	U	61.4
	L	54.7
Optical density dry filter	U	70.7
	L	56.0
Dry wt, filter	U	40.5
	L	42.0
Dry wt, centrifuge	U	27.9
	L	42.8

TABLE 3. Results of paired *t*-tests of differences between means of samples obtained by centrifugation and by filtration.

Variables	df	*t* value	Difference between means	95% confidence interval of difference
Upper depth				
O.D. wet filter vs. O.D. dry filter	13	5.714	.3294	.2049–.4539
Dry wt filter vs. dry wt centrifuge	14	6.574	14.4733	9.7512–19.1954
Lower depth				
O.D. wet filter vs. O.D. dry filter	13	6.255	.2844	.1862–.3226
Dry wt filter vs. dry wt centrifuge	14	6.716	14.4267	9.8193–19.0341

t 99, 13 df = 3.012
t 99, 14 df = 2.977

Discussion — Values tended to increase over time (Fig. 1 and 2); curves from values at the lower depth were essentially the same as those from the upper, so are not reproduced. Amounts of seston were probably influenced by changes in planktonic species and by fluctuations in water level. Microscopic examination revealed the earlier samples to be dominated by diatoms, green algae, and zooplankters. Later samples contained a preponderance of blue–green monerans. Water level was high in early June and decreased to a minimum July 12. On July 18 refilling began; thereafter the level fluctuated as water was simultaneously withdrawn and run in. Influx predominated and the level rose slowly from July 18 to last sampling.

Maximum dry weights coincided with the lowest water level; maximum optical density was recorded after refilling began. Curves showed general

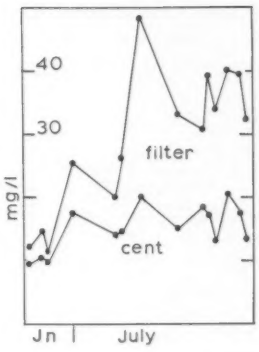

FIG. 1. Filter and centrifuge (cent) estimates of standing crop of seston in the upper 1.5-m stratum of Dixon Reservoir, Colorado. Each point is the average of two values.

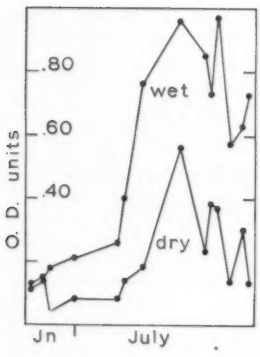

FIG. 2. Acetone extract estimates of standing crop of seston in the upper 1.5-m stratum in Dixon Reservoir, Colorado. Each point is the average of two values from wet or dry filter samples.

correspondence in high and low points, suggesting that filter and centrifuge were sensing the same changes in seston.

We did not achieve complete solution of pigments, as indicated by a greenish or more frequently, a bluish-green color remaining on wet filters after extraction. Possibly grinding or ultrasonic disruption of cells would have removed this problem. Dried filters often appeared brownish after extraction; whether due to inorganic matter or oxidized pigments is not known.

To apportion variation associated with time, depth, and method, the data were subjected to analysis of variance (Table 4). Time was of much greater importance than depth for filter variables, but effect of depth was more important than time for centrifuge samples.

Relations between optical density and dry weight of filter samples were examined by correlation and regression. Dry weight was significantly correlated at the 1% level with wet optical density at both upper and lower depths (r = .828 and .667, respectively, N = 15). Dry weight was not significantly correlated with optical density determined from dried filters at either depth (r = .498 and .324, N = 14). The regression of seston dry weight on optical density for the upper stratum (Fig. 3) was described by $\hat{Y} = 13.21 + 30.92\ X$.

TABLE 4. Analysis of variance, estimates of seston crops obtained by filtration with glass fiber discs, and centrifugation.

	Source of variation	df	Mean square	F
Wet optical density filter	Depth	1	.0653	30.02**[a]
	Date	14	.2998	137.76**
	Interaction	14	.0170	7.79**
	Error	30	.0022	
Dry wt filter	Depth	1	291.7215	16.51**
	Date	14	537.7253	30.43**
	Interaction	14	133.4957	7.55**
	Error	30	17.6688	
Dry wt centrifuge	Depth	1	298.8202	10.18**
	Date	14	106.2267	3.62**
	Interaction	14	59.8880	2.04*
	Error	30	29.3361	
Dry optical density filter	Depth	1	.0101	11.98**
	Date	13	.7847	928.25**
	Interaction	13	.0057	6.71**
	Error	28	.0008	

[a]** = significant 1%; * = significant 5%.

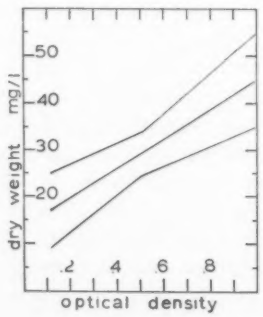

FIG. 3. Regression line with 95% confidence limits, dry weight, and optical density estimates from filtered samples of seston, Dixon Reservoir, Colorado. Optical density from wet filter samples.

Pennak (1949) added 25% to compensate for seston passing through the Foerst centrifuge. A similar correction applied to the mean centrifuge catches in this study gives values of 18.2 and 24.8 mg/liter as estimates of mean crops of seston in the upper and lower depths. Mean filter estimates are 29.0 and 33.4 mg/liter values, 59 and 35% greater.

In summary, we found that gravimetric estimates of standing crop of seston from samples filtered through glass fiber discs were significantly greater than those from centrifuged samples. Filtration is quicker and requires less sample water than centrifugation.

Acknowledgments — Statistical analyses were performed by the Computer Center, Colorado State University. We thank Drs P. Mielke and B. Dowden for their assistance. D. F. Reed was a participant in N.S.F. Participation for High School Teachers Program, Colorado State University.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION. 1965. Standard methods for the examination of water and wastewater. 12th ed. APHA, Inc., New York. xxxi + 769 p.

CORCORAN, E., J. KIMBALL, AND J. ALEXANDER. 1964. An improved method of filtration for chlorophyll analyses. Bull. Mar. Sci. Gulf Caribbean 14: 545–548.

HARTMAN, R. T. 1958. Studies of plankton centrifuge efficiency. Ecology 39: 374–376.

KEREKES, J. 1966. Efficiencies of the net plankton and the centrifuged seston methods in highly eutrophic lakes. J. Fish. Res. Bd. Canada 23: 1625–1628.

PENNAK, R. W. 1949. Annual limnological cycles in some Colorado reservoir lakes. Ecol. Monogr. 19: 233–267.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1960. A manual of sea water analysis. Bull. Fish. Res. Board Can. 125. vi + 185 p.

WELCH, P. S. 1948. Limnological methods. The Blakiston Co., New York. xviii + 381 p.

Integrating Radiometer: A Self-Contained Device for Measurement of Submarine Light Energy in Absolute Units[1]

T. PLATT

Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute, Dartmouth, N.S.

AND

E. LARSEN AND R. VINE

Department of Energy, Mines and Resources
Atlantic Oceanographic Laboratory
Bedford Institute, Dartmouth, N.S.

PLATT, T., E. LARSEN, AND R. VINE. 1970. Integrating radiometer: a self-contained device for measurement of submarine light energy in absolute units. J. Fish. Res. Bd. Canada 27: 185–191.

The design, construction, and use of a self-contained submarine radiometer is described. The instrument measures the time-integral of the downwelling irradiance at a given depth in absolute units. The measurement error at 20 m in turbid coastal water is less than 5%. The instrument is useful in primary production and heat budget studies, and could find many applications in terrestrial ecology.

Received October 7, 1969

[1]Bedford Institute Contribution No. 171. Canadian Contribution to the International Biological Programme No. CCIBP-47.

Printed in Canada (J1611)

CONVENTIONAL METHODS for estimation of submarine light levels involve calculation using data on surface radiation and water transparency. The result is subject to error because of uncertainty in the spectral distribution of the incident radiation, the correction for reflection and other losses at the sea surface, and in the wavelength dependence of the absorption coefficient. All of these difficulties would be obviated if one used an in situ device whose response was independent of the wavelength of the incident light. The thermopile is a suitable transducer and it can be calibrated in absolute units. Thermopiles have been used as detectors in a two-cell transparency meter (Stephens and Strickland, 1962), but their sensitivity is not great, so that at the generally low light levels encountered even near the surface of the sea, they are inadequate for *instantaneous* measurements. However, this objection does not apply to the measurement of light energy integrated over a period of time, and it is this kind of measurement that has wide application in oceanography, particularly in primary production and heat budget studies, where one wants to know the *total* light energy incident in a given period. Further, the instrument should be self-contained and therefore battery-operated, so that the ship can be free to carry out other duties during the period of exposure. A self-contained integrating radiometer has been built previously (Draper, 1961) but this device used a photocell as transducer, and the spectral response was therefore not flat. In this note we describe the design, construction, and use of an integrating underwater radiometer using a thermopile.

DESIGN CONSIDERATIONS

Optical collector — As collector, we have used a hollow hemisphere of Schott WG7 clear glass 50 mm in diameter and 2 mm thick, purchased, as was the thermopile, from the Eppley Laboratory, Newport, Rhode Island. The thermopile detector is located at the centre of this hemisphere, and in its equatorial plane. The instrument, therefore, measures only the downwelling irradiance. The upwelling irradiance is generally small compared with the downwelling component (Jerlov, 1968). Since we have used no flat optical filters, we have avoided the need to diffuse the incident light as was done in the radiometer made by Draper (1961), which also employed hemispherical collectors. However, the present design could be modified for selective wavelength transmission either by using a coloured Schott glass hemisphere or by using double concentric hemispheres of clear glass enclosing liquid filters as was done in the design of the UVB photometer by Jerlov (1951).

Spectral window — The Schott WG7 glass is transparent in the wave length range from 285 to 2800 mμ. For primary production studies it is customary to measure only that radiant energy within the "photosynthetic range" ($\sim$350-720 mμ.) There is little or no direct solar radiation at wavelengths less than $\sim$300 mμ and the extinction coefficient for sea water is greater than unity for wavelengths greater than $\sim$750 mμ and increases rapidly at higher wavelengths (Strickland, 1958). Therefore, if the measurements are to be confined to the range 350–750 mμ we are faced with a problem in the range

300-350 mμ, for which sea water is quite transparent. It can be calculated from the data in the Smithsonian Tables (List, 1963) that of all the solar radiant energy in the photosynthetic waveband, only 5% is in the range 300–350 mμ. Hence, the bias would be small, but in any case it could be argued that photosynthetic efficiencies should be calculated on the basis of what energy is *available*, rather than what energy is actually *utilized*. Of course, these difficulties would not arise if the instrument were used for heat budget studies.

Integrating device — Two different types of integrating readouts have been tried. First, the Curtis Model 150-SP, meter (Curtis Instrument Inc., Mount Kisco, N.Y.), which depends on the plating of mercury across an electrolytic gap. The gap displacement, which is measured by means of a travelling microscope, is a measure of the time-integral of the applied current. Second, the Bisset Berman E cell Type S-114 (Bisset Berman Corp. Los Angeles, California), which depends on the plating of silver across an electrolyte onto a central electrode. The accumulated charge, which is proportional to the time-integral of the applied current is measured by deplating using a Bisset Berman Model 300 EDR readout. Most of our work has been done with the Curtis meter, but the second device is probably better in that the associated amplifier circuit (see below) consumes less power, is more sensitive, and has a more precise readout system.

Amplifier — The sensitivity of the Eppley model F4 thermopile is ~5 mV cal^{-1} $cm,^{-2}$ so that when it is exposed to the low light levels in the sea, the voltage generated is quite small, and needs to be amplified before it is integrated. Since the signal is direct current, some form of chopper stabilization is required. Design of the amplifier depends on the maximum readout error that can be tolerated. The initial specifications called for a maximum error of 5% over a depth range of 0–20 m in water of extinction coefficient not greater than 0.2. Of the two most feasible designs for the amplifier circuit, it was calculated that the configuration shown in Fig. 1B is less susceptible to interference noise than that shown in Fig. 1A, and the former was therefore employed. A simple calculation showed that the effect of a possible error introduced in this configuration by the Joule effect was negligible.

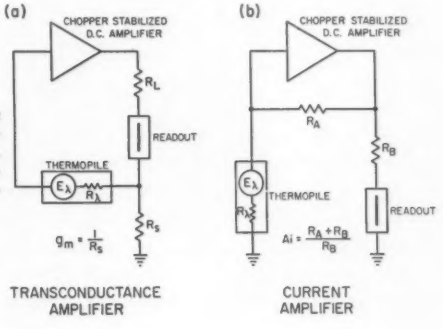

FIG. 1. Showing the two most feasible configurations for the amplifier: (*a*) transconductance amplifier; (*b*) current amplifier. It was calculated that of these two, configuration (*b*) was less susceptible to interference noise.

For the Curtis meter, where the required gain was ~100 db, a Zeltec 148 amplifier (Zeltec Inc., Concord, California) was used (Fig. 2). For the E cell, where the required gain was only ~75 db, a hybrid amplifier was designed specially, which used much less power than the Zeltec model, and had a lower initial cost (Fig. 3).

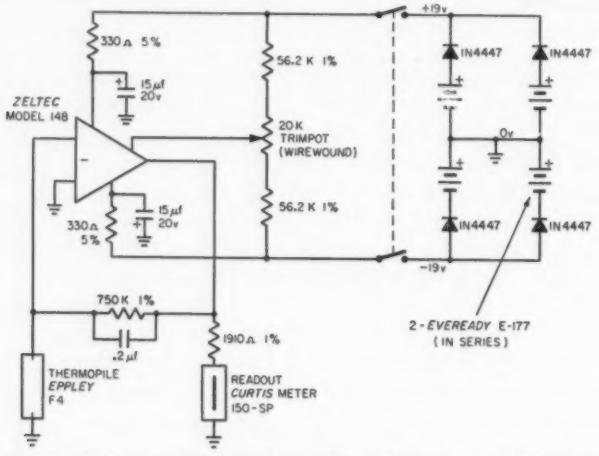

FIG. 2. Schematic diagram of amplifier circuit for use with Curtis meter integrating readout.

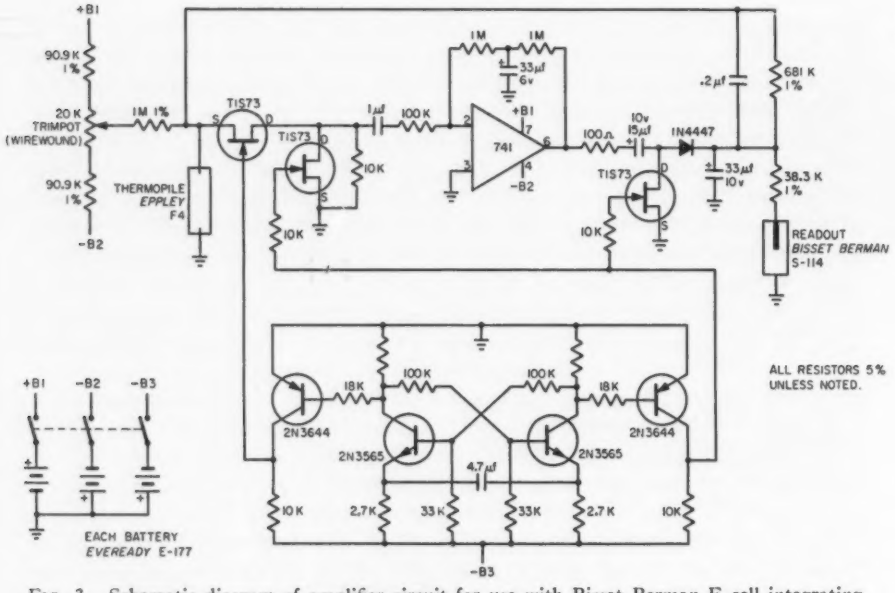

FIG. 3. Schematic diagram of amplifier circuit for use with Bisset Berman E cell integrating readout.

Of the three components of offset drift (temperature, time, and battery voltage), that due to temperature change is the most important by an order of magnitude. In the interests of design simplicity, we have preferred not to include internal temperature compensation, but rather to determine the variation of offset drift as a function of temperature and use this information to correct the results for temperature. The temperature offset is roughly constant between 0 and 6 C. Between 6 and 16 C, it decreases linearly. It is essentially zero between 16 and 30 C. The maximum effect of temperature would be equivalent to an error in reading the gap displacement of $\sim 6 \times 10^{-3}$ cm. This effect would be significant only at extremely low light levels. The silver oxide alkaline-type batteries (Eveready #E177) used for both models were chosen for their relatively constant supply voltage as a function of time at low temperatures. A more detailed analysis of the circuitry is given in Larsen (MS, 1969).

Pressure case — The pressure case is made from a section of anodized aluminium pipe 2.375 inches (6.03 cm) diameter and 0.15 inches (0.38 cm) thick (Fig. 4). The complete instrument weighs about 5 lb (2250 g). The glass hemisphere is cemented into a groove in the instrument head by an epoxy resin (100 parts Hysol 2039 resin, 25 parts Hysol 3475 hardener; Hysol Canada Ltd., Toronto). This mixture has the advantage of remaining slightly flexible after hardening so that differential thermal expansion between glass and aluminium does not result in a fracture of the glass.

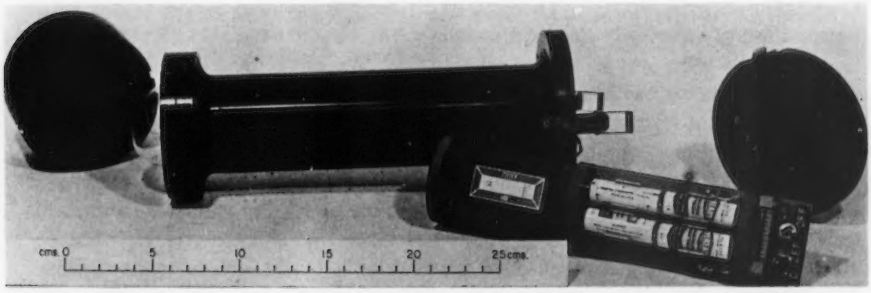

FIG. 4. Physical arrangement of radiometer, showing from left to right the light-tight cover with sponge rubber insert to protect the glass hemisphere; the pressure case; the electronics module with amplifier, power supply, Curtis meter, and zero adjust; end cap for pressure case with O-ring seal.

CALIBRATION

The instrument was calibrated in air against an Eppley 180° pyranometer. The output from the pyranometer was followed on a potentiometric chart recorder and the total incident energy during the exposure period was found by planimetric integration of the chart record. The light energy measured by the submarine radiometer is expressed in calories. The calibration was found to be linear throughout the range of daylight intensities encountered.

An independent check on the linearity of the amplifier circuits themselves showed that they were both quite linear throughout their dynamic ranges.

Calibration every 12 months is sufficient. Battery life is ~30 hr using the Curtis Meter and ~80 hr using the E cell. The effect of battery aging is corrected by an offset adjustment using a simple centre-zero galvanometer.

The precision of the radiometer declines at light levels low enough that the inherent noise is no longer small compared with the signal generated by the thermopile. With the present design we calculate that the 5% readout error would be exceeded at depths greater than 20 m in water of extinction coefficient 0.2, with surface radiation ~10 cal cm^{-2} hr^{-1}. In more transparent waters, the maximum useful depth would be correspondingly increased. At light levels where the thermopile signal is large compared with the interference noise, the precision is limited by the resolution of the readout device, which will be greatest at deflections close to full scale. The design has been optimised for use from 0 to 20 m under the condition specified above.

USE OF THE RADIOMETER

The radiometer has been used for both field and laboratory applications. In the field, it has been used to measure submarine light energy integrated over a period of 4 hr in conjunction with in situ ^{14}C primary production experiments. This allows the direct calculation of energy efficiency of photosynthesis (Platt, 1969). The instrument is simple to use. The bottles for the production experiment are clamped to a length of hydrographic wire suspended from a buoy. The radiometer is clamped to this wire in such a manner that it is held out from the wire and in a vertical position. We have used it so far only in inshore waters; for use in rougher seas, further precautions might be necessary to ensure that the instrument remained vertical.

The minimum permissible exposure time depends on the light levels at the depth of exposure. In designing to meet the criterion of less than 5% error at 20 m, we have assumed an exposure time of 4 hr, which is the standard time for our ^{14}C experiments. Lower light levels (or shorter times) could be measured if either a more sensitive readout or a better amplifier were used. The amplifiers used here represent the state-of-the-art for a reasonably inexpensive device.

In general, we have found good agreement between in situ light readings and light levels computed from surface radiation and transparency data, but we have found some indication that on very bright days the correction factor for surface loss should be somewhat greater than the figure of 5% that is commonly used (Strickland, 1958). With a surface radiation of ~10 cal cm^{-2} hr^{-1} we have measured the radiation at 5 m in St. Margaret's Bay, N.S., to be ~2 cal cm^{-2} hr^{-1} and in the Bedford Basin, Nova Scotia to be ~0.65 cal cm^{-2} hr^{-1}. These figures are consistent with the difference in extinction coefficients between the two places; the coefficient is ~0.14 in St. Margaret's Bay and ~0.34 in the Bedford Basin.

In the laboratory we have used this radiometer for the calibration of the illuminators inside the water bath of Warburg manometer apparatus for the measurement of the photosynthetic rate of phytoplankton cultures. There is no reason why the radiometer could not be used for light energy measurements in terrestrial ecology.

Acknowledgments — It is a pleasure to thank Mr S. B. McPhee for his co-operation. We thank also Mr C. A. Godden for advice on the choice of an epoxy resin and Mr B. Irwin for assistance in the field measurements. We acknowledge the contribution of Messrs J. Budlong and W. B. Fraser in early stages of the project.

REFERENCES

DRAPER, L. 1961. Self-contained integrating irradiance meter for use underwater. J. Sci. Instrum. 38: 474–476.

LARSEN, E. MS, 1969. Design of a self-contained submersible integrating radiometer. Atlantic Oceanographic Laboratory, Bedford Institute Report #1969-8, Dartmouth, N.S.

JERLOV, N. G. 1951. Optical studies of ocean waters. *In* H. Petersson [ed]. Reports of the Swedish Deep Sea Expedition (1947–8), Vol. III. p. 1–60.
1968. Optical oceanography. Elsevier Publishing Company, Amsterdam. 194 p.

LIST, R. J. 1963. Smithsonian meteorological tables. 6th ed., revised. Smithsonian Institution, Washington, D.C. xi + 527 p.

PLATT, T. 1969. The concept of energy efficiency in primary production. Limnol. Oceanogr. 14: 653–659.

STEPHENS, K., AND J. D. H. STRICKLAND. 1962. Use of a thermopile radiometer for measuring the attenuation of photosynthetically active radiation in the sea. Limnol. Oceanogr. 7: 485–487.

STRICKLAND, J. D. H. 1958. Solar radiation penetrating the ocean. A review of requirements, data and methods of measurement, with particular reference to photosynthetic productivity. J. Fish. Res. Bd. Canada 15: 453–493.

An Inflammatory Lesion in an American Shad, *Alosa sapidissima*

RONALD W. WARNER AND STANLEY C. KATKANSKY

Marine Resources Region
California Department of Fish and Game
Menlo Park, Calif. 94025, USA

WARNER, R. W., AND S. C. KATKANSKY. 1970. An inflammatory lesion in an American shad, *Alosa sapidissima*. J. Fish. Res. Bd. Canada 27: 191–193.

An adult male American shad, *Alosa sapidissima*, with an external swelling simulating a neoplasm was collected from the Russian River, California. Histological examination of the large cone-shaped protuberance disclosed it to represent chronic inflammation and repair in response to injury and infection. The combined findings suggest the lesion may have originated from a lamprey bite.

Received October 3, 1969

Printed in Canada (J1609)

RECORDS OF TUMORS and tumorlike growths in fish are well documented. Schlumberger and Lucké (1948), Nigrelli (1953), van Duijn (1967), and Wellings (1969) give excellent reviews and compilations of abnormalities in many species of fish.

An American shad, *Alosa sapidissima*, with a tumorlike growth on its side, was collected from the Russian River, near Guerneville, California in May 1968. Because of the absence of any records of abnormal growths in American shad, it seemed appropriate to examine the specimen in detail and to report our observations.

The shad, which on dissection proved to be a male, measured 49.5 cm total length. Tissue blocks from the lesion were photographed and then fixed in Davidson's solution (Shaw and Battle, 1957). The tissue was processed, using standard histological techniques. Cross sections (7 μ thick) were stained with hematoxylin and eosin, Mallory's aniline blue collagen stain, Giemsa stain, and the MacCallum–Goodpasture stain for bacteria in tissue.

Located on the left side, dorsolateral to the anal fin, the cone-shaped protuberance displayed a central ulceration with a dark red, granular mass extending beyond the surrounding epithelium (Fig. 1). Gross examination of the lesion disclosed firm, glistening, white tissue heavily vascularized in the central part. In the area of its greatest width (36 mm), the lesion almost completely displaced the hypaxial muscle mass in that portion of the trunk (Fig. 2).

Histological examination of the lesion revealed dense layers of collagen interspersed with highly disorganized striated muscle bundles (Fig. 3). The lesion was well vascularized (Fig. 4), with infiltrates of plasma and lymphoid cells occupying the perivascular areas predominately. Tissue closely resembling granulation tissue in human wounds comprised much of the superficial portion of the lesion. Most of the remaining areas of the lesion had undergone the final reparative process of scar formation. Deposits of hemosiderin were present in some of the more vascularized areas and numerous colonies of gram-negative bacteria were scattered throughout the tissue in spaces between collagen fibers and muscle bundles (Fig. 5). Figure 6 illustrates the general structural relationship of the major components of the lesion.

The gills, viscera, and skeletal systems had no grossly evident abnormalities.

This lesion most closely resembled chronic inflammation and repair in response to an injury. The shape of the large tissue defect and the extensive chronic inflammatory response suggest that the lesion may have been caused by a lamprey bite. Combined effects of prolonged mechanical damage by buccal teeth and lytic action of lampredin on exposed flesh could have induced the extensive fibrosis. Mechanical injury from other than biological sources might also be implicated as a cause.

The diffuse infection by bacteria may have been instrumental in producing the intense connective tissue response. Nigrelli (1953) states that a heavy infection of myxosporidians in subcutaneous tissues of fish often results in large swellings resembling neoplasia. In a juvenile American shad, Haley et al.

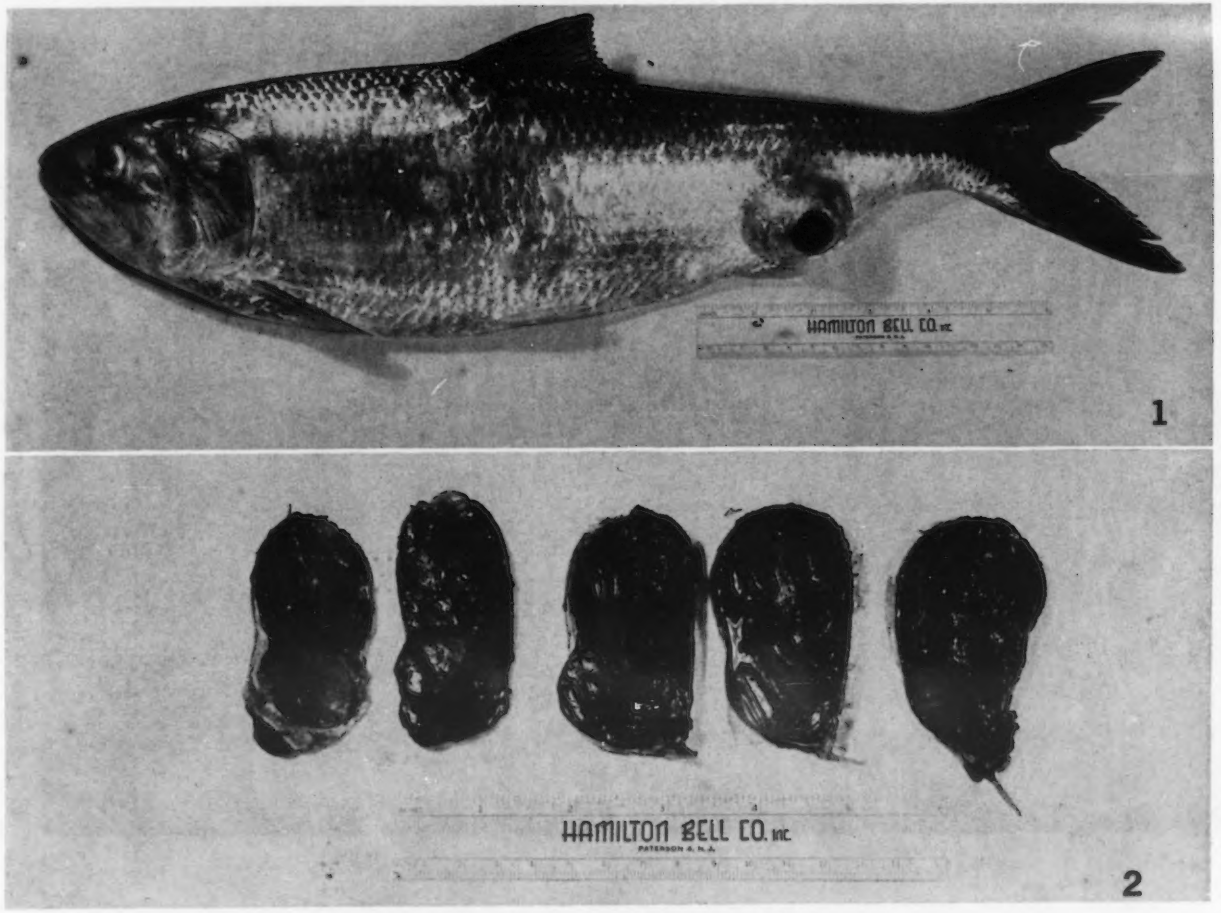

FIG. 1. American shad, with an ulcerated lesion.
FIG. 2. Gross sections of lesion, showing displacement of hypaxial muscle mass.

FIG. 3. A section of the lesion, showing dense collagen deposits and disarranged muscle bundles. C = collagen; M = muscle bundles; B = bacterial colonies.

FIG. 4. A section of the lesion, showing a well-vascularized area. B = blood vessel.

FIG. 5. Detail of a section of the lesion, displaying numerous bacterial colonies. B = bacterial colonies.

Warner and Katkansky — J. Fish. Res. Bd. Canada

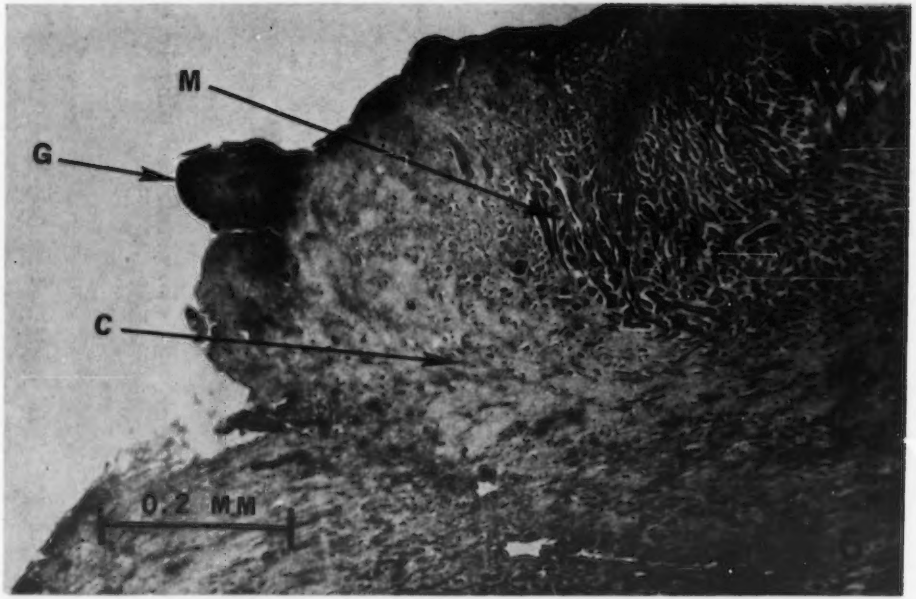

FIG. 6. General structural relation of the major components of the lesion. G = granulation tissue; M = muscle; C = collagen.

(1967) found subdermal lesions resembling those associated with fish furunculosis. The bacterium *Aeromonas liquefaciens* was isolated from these lesions. Since we are uncertain of the agent responsible for the abnormality reported here, it is possible that a bacterial infection alone could have provided the necessary stimulus for inflammation and increased deposition of collagen, producing the conical protuberance. However, histological evidence of elements characteristic of chronic inflammation and wound repair support the interpretation that this abnormality was the result of an injury, with the extensive collagenization being a compensatory response to the loss of tissue. The bacterial colonies were in all likelihood secondary invaders.

It is purely speculative that the lesion resulted from a lamprey bite. Support for this concept could be obtained through a study of the response to lamprey bites at serial intervals after the time of attachment of the parasite. Such a study would provide basic information on wound healing and collagen production in fishes, and might lead to discovery of criteria that would permit positive identification of lamprey bites of recent origin.

Acknowledgments — The authors wish to thank C. J. Dawe, M.D., of the Pathology Laboratory at the National Cancer Institute for assistance in interpreting the nature of the lesion and for critical evaluation of the manuscript. We extend our appreciation to R. Haley, California Department of Fish and Game, for his encouragement.

REFERENCES

DUIJN, C. VAN JR. 1967. Diseases of fish. Charles C. Thomas, Springfield, Illinois. 309 p.

HALEY, R., S. P. DAVIS, AND J. M. HYDE. 1967. Environmental stress and *Aeromonas liquefaciens* in American and threadfin shad mortalities. Progr. Fish-Cult. 9: 193.

NIGRELLI, R. F. 1953. Tumors and other atypical cell growths in temperate freshwater fishes of North America. Trans. Amer. Fish. Soc. 83: 262–296.

SCHLUMBERGER, H. G., AND B. LUCKÉ. 1948. Tumors of fishes, amphibians, and reptiles. Cancer Res. 8: 657–754.

SHAW, B. L., AND H. I. BATTLE. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Can. J. Zool. 35: 325–327.

WELLINGS, S. R. 1969. Neoplasms and primitive vertebrate phylogeny: echinoderms, prevertebrates, and fishes — a review. J. Nat. Cancer Inst. Monogr. 31: 59–128.

Chloride Regulation in the Burrowing Worm Eel *Moringua linearis*

A. SUBRAMANIAN

Marine Biological Station, Porto Novo, Madras, S. India

SUBRAMANIAN, A. 1970. Chloride regulation in the burrowing worm eel *Moringua linearis*. J. Fish. Res. Bd. Canada 27: 193–196.

The concentration of chloride in the blood of *Moringua linearis* (Gray) varied within a narrow range in association with wide fluctuations of salinity in both the natural environment and the laboratory.

Received June 9, 1969

Printed in Canada (J1501)

THE BURROWING WORM EEL *Moringua linearis*, by nature of its occurrence in estuarine mud flats, encounters a wide range of salinity. Parry (1966) has indicated that the physiological responses of fish to changing salinity are easily measured by following changes in blood concentration. Accordingly, the present investigation was made to determine the chloride concentration in the blood of *M. linearis* in association with wide fluctuations of salinity in the natural environment and the laboratory.

Whole blood was collected from an incision made in the tail after the fish had been washed thoroughly in distilled water and wiped clean with a filter paper. Sendroy's method as modified by Vanslyke and Hiller (Hawk et al., 1954) was used to estimate chloride concentration of the heparinized blood.

Thirty-eight observations on concentrations in the natural habitat were made at intervals of 10 days during 1967 in animals collected at low tide. The interstitial salinity at the time of collection was noted.

For laboratory studies, eels were adapted separately in different salinities viz., 0.4‰, 8.2‰, 16.8‰, 24.1‰, and 32.2‰. An adaptation period of 10 days was allowed in each medium before blood was taken for analysis. The animals were kept at room temperature (29 ± 1 C) and not fed during the period of adaptation. Six samples were analysed for each salinity.

Only eels of the same size (4.25–4.67 g) and sex (female) were selected for the whole investigation.

An attempt was made to compare the results of laboratory and field investigations from the regression lines of blood chloride on salinity. Covariance analysis was carried out to determine the level of significance.

The salinity of seep water in the holes from which eels were collected varied between 0.5‰ and 29.0‰ during the year (Fig. 1). It increased gradually in summer and declined during the onset of rain. However, variation was especially marked in July and November. This would have resulted because of the influence of tide and variation in freshwater flow.

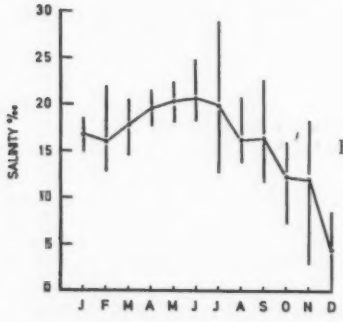

FIG. 1. Salinity fluctuations (means and ranges) in the habitat of *Moringua linearis* in 1967.

In both the natural habitat and the laboratory the eels maintained the chloride concentration within a narrow range in spite of wide fluctuations in salinity (Fig. 2). In the natural habitat the concentration varied from 194.3 to 224.5 mEq/liter in association with salinities ranging from 0.5 to 29.0‰.

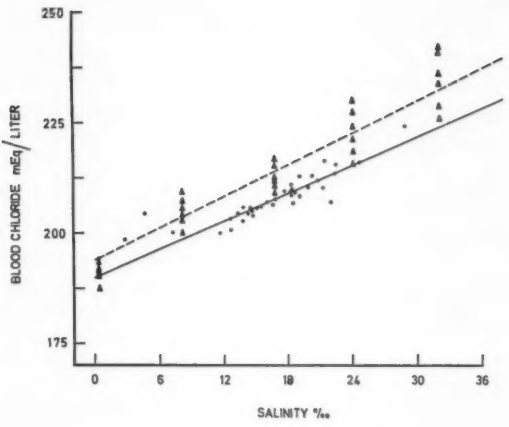

FIG. 2. Chloride concentrations in the natural habitat (●) and the laboratory (▲) conditions. Solid and broken lines are regression lines.

In the laboratory the mean concentration varied from 191.2 to 232.6 mEq/liter when the salinity range was from 0.4 to 32.2‰. The concentration decreased slightly in lower salinities and increased in higher salinities. Thus *M. linearis*, like steelhead (Houston, 1959) and brown (Gordon, 1957) trout, maintains the blood chloride at two different levels, one characteristic of freshwater medium and the other slightly higher level characteristic of seawater medium.

The statistical analysis showed that the chloride concentrations for a given salinity differed significantly in the laboratory and under natural conditions. This difference would have resulted because of the natural fluctuations in salinity in the estuary. Moreover, it will be necessary to study in detail the temperature conditions and the rate of adaptation to salinity change in nature since temperature (Wikren, 1953; Gordon, 1959; Selvarajan, 1962; Hickman et al., 1964; Rao, 1969) and rate of adaptation (Keys, 1933; Black, 1951; Houston, 1959; Parry, 1960) are known to influence the blood concentration in fishes.

Acknowledgments — I am grateful to Prof R. V. Seshaiya, Director, Marine Biological Station, Annamalai University, Porto Novo, for his encouragement in the work; and to Prof W. T. W. Potts, Department of Biology, University of Lancaster, Lancaster, England, for criticism of the manuscript.

REFERENCES

BLACK, V. S. 1951. Some aspects of the physiology of fish II. Osmotic regulation in teleost fishes. Univ. Toronto Stud., Biol. Ser. 59, Publ. Ont. Fish. Res. Lab. 71: 53–89.

GORDON, M. S. 1957. Observations on osmoregulation in the arctic char (*Salvelinus alpinus* L.). Biol. Bull. (Woods Hole) 112: 28–33.
1959. Ionic regulation in the brown trout (*Salmo trutta* L.). J. Exptl. Biol. 36: 227–252.

HAWK, P. B., B. L OSER, AND W. H. SUMMERSON. 1954. Practical physiological chemistry. 13th ed. McGraw Hill Book Co. Inc., New York. p. 627–629.

HICKMAN, C. P., JR., R. A. MCNABB, J. S. NELSON, E. D. VAN BREEMEN, AND D. COMFORT. 1964. Effect of cold acclimation on electrolyte distribution in rainbow trout (*Salmo gairdneri*). Can. J. Zool. 42: 477–597.

HOUSTON, A. H. 1959. Osmoregulatory adaptation of the steelhead trout (*Salmo gairdneri* Richardson) to sea water. Can. J. Zool. 37: 729–748.

KEYS, A. B. 1933. The mechanism of adaptation to varying salinity in the common eel, and the general problem of osmotic regulation. Proc. Roy. Soc. Ser. B Biol. Sci. 112: 184–199.

PARRY, G. 1960. The development of salinity tolerance in the salmon *Salmo salar* L. and some related species. J. Exptl. Biol. 37: 425–434.
1966. Osmotic adaptation in fishes. Biol. Rev. 41: 392–444.

RAO, G. M. M. 1969. Effect of activity, salinity and temperature on plasma concentrations of rainbow trout. Can. J. Zool. 47: 131–134.

SELVARAJAN, V. R. 1962. Chloride regulation in a fresh water fish, *Cirrhina reba* under heterosmotic conditions and high temperature. Proc. Indian Acad. Sci. Sect. B 55: 91–98.

WIKREN, B. J. 1953. Osmotic regulation in some aquatic animals with special reference to the influence of temperature. Acta Zool. Fenn. 71: 1–102.

Effects of Some Environmental Factors on Survival, Condition, and Timing of Emergence of Chum Salmon Fry (*Oncorhynchus keta*)[1]

L. M. DILL[2]

Department of Fisheries and Forestry of Canada
Vancouver, B.C.

AND

T. G. NORTHCOTE

Institute of Animal Resource Ecology
University of British Columbia, Vancouver, B.C.

DILL, L. M., AND T. G. NORTHCOTE. 1970. Effects of some environmental factors on survival, condition, and timing of emergence of chum salmon fry (*Oncorhynchus keta*). J. Fish. Res. Bd. Canada 27: 196–201.

In an experiment in incubation channels at Robertson Creek, B.C., survival of chum salmon from planting of eggs to emergence of fry was higher in large gravel (2–4 inches, 5.1–10.2 cm) than in small gravel (0.4–1.5 inches, 1.0–3.8 cm). Neither condition coefficient nor timing of emergence was affected by gravel size. There were no significant effects of egg burial depth (8 and 12 inches, 20.3 and 30.5 cm) or density (50 and 100 per treatment) on condition coefficient, or timing of emergence.

Received May 29, 1969

[1]Based on a thesis submitted by L.M.D. in partial fulfillment of the requirements for the degree of Master of Science at the University of British Columbia, Vancouver, B.C.

[2]Present address: Institute of Animal Resource Ecology, University of British Columbia, Vancouver, B.C.

Printed in Canada (J1489)

ALTHOUGH IT HAS BEEN SHOWN that gravel size (Phillips and Campbell, 1962; Phillips, 1964; McNeil and Ahnell, 1964; McNeil, 1966), depth of egg burial (Shelton, 1955), and egg density (McNeil, 1969) are important in determining subgravel survival of salmonid eggs and alevins, and that these same factors may affect fry size at emergence (McNeil, 1963) and timing of emergence (Shelton, 1955), these factors have not previously been tested together in any species. In this study two treatments for each factor were examined experimentally in an attempt to determine that combination most favourable for the production of chum salmon fry.

Methods — The experiment was conducted at the Robertson Creek station of the Department of Fisheries and Forestry of Canada, on Vancouver Island near Alberni, B.C. Facilities included a series of small incubation channels whose flows could be equalized by stoplogs.

Two adjacent channels were chosen for the study and eight traps modified from a design used by the Oregon State Game Commission (E. N. Claire, personal communication) were installed in each. The traps (Fig. 1) consisted of five concentric rings of fiberglass screen placed at 0.5-ft

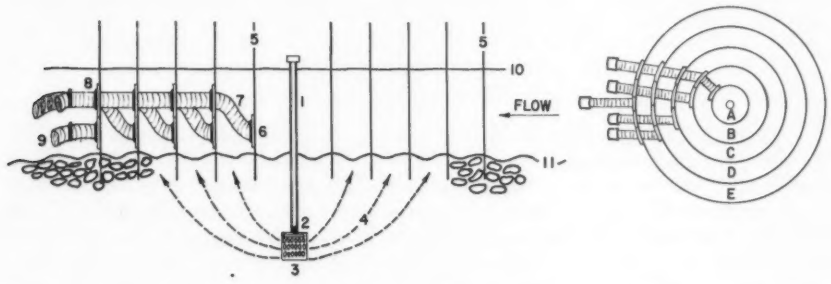

FIG. 1. Elevation (*left*) and plan (*right*) of the concentric ring traps installed at Robertson Creek, B.C.: (1) $\frac{3}{4}$-inch (1.9-cm) P.V.C. standpipe, through which eggs deposited; (2) rubber stopper at base of standpipe, to prevent fry exit; (3) moulded plastic Vibert box to retain eggs but pass alevins; (4) routes of alevin migration through the gravel; (5) concentric rings of 18 × 16 mesh/inch (7.1 × 6.3/cm); (6) entrance to duct via metal sleeve; (7) dryerflex hose duct, 4 inch (10.2 cm) diam; (8) duct passing through 3-inch (7.6-cm) diam hole in $\frac{3}{16}$-inch (0.5-cm) neoprene rubber skirt; (9) cotton bags with mesh ends, one per duct; (10) water surface; (11) gravel level.

(15.3-cm) intervals and supported by lathes. Each ring, therefore, had a diameter 1 ft (30.5 cm) larger than the one within it, the total diameter of each trap being 5 ft (1.5 m). The screens extended 2 inches (5.1 cm) into the gravel and 1.5 ft (45.7 cm) above the water surface. The water was 1 ft (30.5 cm) deep. The rings within the traps facilitated study of lateral fry movement during emergence. Results of that portion of the study are reported elsewhere (Dill, MS, 1967).

Each of the three environmental factors was studied at two treatment levels to provide a 2^3 factorial design. One channel was filled with small gravel (0.4–1.5 inches, 1.0–3.8 cm) and one with large gravel (2–4 inches, 5.1–10.2 cm). Tests for egg depth and density were duplicated within each channel. Gravel was placed in the channels in January 1967. Eyed chum salmon eggs from the Qualicum River were buried in the traps on February 17, by gently pouring them down 1.9-cm diam plastic standpipes into Vibert boxes (5.6 × 3.9 × 6.7 cm) that had been partially filled with gravel and buried in the channels the previous day. The eggs were deposited at either 8- or 12-inch depths (20.3 or 30.5 cm) and in numbers of 50 or 100 eggs per box. The standpipes were sealed with rubber stoppers at the points where they entered the boxes. After hatching the alevins were able to leave the Vibert boxes through slots in the sides.

The standpipes were used to withdraw water samples from the gravel for dissolved oxygen analysis just before (April 27) and just after (June 11) the emergence period. Oxygen concentrations were close to saturation on both dates.

A series of ducts and collection bags was installed to capture the fry emerging from each section of the trap. However, fry could not be captured in this manner, either because they failed to locate the duct entrances or because water velocity was insufficient to retain them in the collection bags. The fry were therefore removed from the traps with a dipnet, sometimes several days after they had emerged.

It was not possible to determine survival within each trap because substantial numbers of fry escaped from the traps during emergence. These were captured on V-screens located at the downstream end of each channel and could thus be assigned only to gravel size treatment. The capture efficiencies of these screens (3.2 mm diam perforations), estimated from samples of 100 fish dyed with Bismarck brown and released in each channel during the peak of migration, were 71 and 34% for the small and large gravel channels respectively. At the termination of the experiment each Vibert box was removed and the contents examined and enumerated.

All fry captured were preserved in 10% formalin for 2 weeks and then weighed to the nearest milligram and measured (fork length) to the nearest millimeter. Weighing was conducted after the fry had been dried at 37 C for 24 hr. The mean weight and mean length of the fry captured within each trap were then used to determine a mean condition coefficient from the following formula, modified from Lagler (1956):

$$k = \frac{\text{mean weight (g)} \times 10^6}{\text{mean length}^3 \text{ (mm)}}.$$

As it was often impossible to remove all fry from the traps immediately after emergence, the only parameter of emergence timing for which a reliable value could be obtained was the number of days after planting at which the first fry appeared in traps.

Analyses of the effects of the three environmental variables on condition coefficient and emergence timing were conducted by means of the Yates tabular method of factorial analysis (Davies, 1956) at $P = .05$.

Results — The number of fry escaping from the concentric ring traps was estimated from the V-screen captures to be 30 in the small gravel and 562 in the large gravel channel. Addition of these figures to the numbers captured within the traps (156 and 49) gave estimates of survival of 31 and approximately 100% in the small and large gravels respectively. The validity of these figures may be questioned since the testing of V-screen capture efficiencies was not replicated, but higher survival in the large gravel was supported by the fact that only 10 dead eggs were found in the Vibert boxes excavated from the large gravel, and 96 dead eggs and alevins in those from the small gravel. However, 320 fry were unaccounted for in the small gravel. These may have died within the gravel after leaving the Vibert boxes.

Analysis of variance revealed no significant effect ($P = 0.05$) of gravel size, egg burial depth, or egg density on coefficient of condition at emergence of those fry captured within the traps. The coefficients according to treatment were:

No./trap	Egg depth in large gravel		Egg depth in small gravel	
	8 inches	12 inches	8 inches	12 inches
50	1.29	1.42	1.40	1.43
100	1.48	1.37	0.96	1.41

Similarly, there was no significant effect of any of the environmental factors tested on timing of emergence. The numbers of days from planting to the earliest recorded emergence, according to treatment, were:

No./trap	Egg depth in large gravel		Egg depth in small gravel	
	8 inches	12 inches	8 inches	12 inches
50	77.0	78.0	79.0	79.5
100	74.5	79.0	91.0	78.5

Discussion — Condition of fry at emergence could be dependent on gravel size for several reasons. Intra-gravel oxygen concentrations are determined by intra-gravel flow, which depends in part upon the porosity of the gravel bed (McNeil and Ahnell, 1964). Therefore, the larger the gravel interstices, the higher the intra-gravel oxygen concentrations.

Several authors have shown that low oxygen concentrations result in reduced fry size (McNeil, 1963; Shumway et al., 1964; Brannon, 1965; Koski, MS, 1966; Mason, 1969). Phillips (1964) has reported that only the smallest fry are able to emerge from small gravel, thereby compounding this effect.

Two effects of larger gravel could tend to mask those mentioned above. First, the higher intra-gravel flow in the large gravel may result in forced activity and excessive expenditure of energy stores and so decrease the energy available for maintenance and growth, as pointed out by Brannon (1965). Second, in larger gravel, there may be less support for the alevins with their large yolk sacs. Constantly having to right themselves, the alevins may use more energy for maintenance and thus be smaller at emergence than their counterparts in small gravel. Such a relationship has been noted previously by both Marr (1965) and Bams (1969). In the present experiment, different sets of environmental conditions may have acted to reduce the size of fry in both gravel sizes to approximately the same extent.

Survival to emergence could have been dependent on gravel size for three reasons. First, a lower oxygen supply in the small gravel may have resulted in increased mortality (Koski, MS, 1966; McNeil, 1963; Phillips and Campbell, 1962). Secondly, survival may have been decreased by the direct action of silt upon the alevins (Stuart, 1953). Thirdly, survival to emergence may have been low, even if survival to hatching had been high, because the alevins were entrapped within the small gravel and prevented from emerging (Sano, 1955; Koski, MS, 1966; Phillips, 1965).

In the present study, no lack of oxygen could be demonstrated in the small gravel. Reduced egg-to-fry survival was therefore most likely due either to entrapment of the alevins or to alevin mortality as a result of siltation. Silt levels were high in both channels but no comparative figures were obtained in the two gravel sizes.

Timing of emergence was not significantly affected by gravel size, a conclusion also reached by Phillips (1964) for coho salmon and steelhead trout. Shelton (1955), however, reported that chinook salmon emergence began

earlier in smaller gravel, and Koski (MS, 1966) found that an increase in percent of fine sediments decreased the time to first emergence of coho salmon. McNeil (1963), on the other hand, suggests that fry emergence is delayed where environmental factors are most likely to cause oxygen stress during development.

The two egg burial depths tested had no apparent effects on timing of emergence, or coefficient of condition. Shelton (1955), however, found that deep (>6 inches or 15.2 cm) burial in small gravel resulted in lower survival, presumably because emergence was made more difficult. Koski (MS, 1966) suggested that the depth and location of eggs within a redd will affect egg-to-fry survival and the condition coefficient of the fry at emergence.

Egg density had no significant effect on condition coefficient or emergence timing possibly because the highest density tested did not approach a "critical" level.

Acknowledgments — The authors are grateful to the following employees of the Department of Fisheries and Forestry of Canada, who provided assistance at various stages of the program: D. MacKinnon, Chief Biologist; B. Lister and F. J. Fraser, Biologists; and J. Baker, technician. Apparatus construction was facilitated by the assistance of fellow students B. Ayles, R. Brock, B. Davies, R. Nagano, and A. Tautz. The manuscript was critically reviewed by Drs W. S. Hoar, J. D. McPhail, and N. R. Liley.

REFERENCES

BAMS, R. A. 1969. Adaptations of sockeye salmon associated with incubation in stream gravels, p. 71–87. *In* T. G. Northcote [ed.] Salmon and trout in streams. H. R. MacMillan Lectures in Fisheries, Univ. of British Columbia, Vancouver, B.C.

BRANNON, E. L. 1965. The influence of physical factors on the development and weight of sockeye salmon embryos and alevins. Int. Pac. Salmon Fish. Comm., Prog. Rept. 12: 1–26.

DAVIES, O. L. [ed.]. 1956. Design and analysis of industrial experiments. Hafner, N.Y. 636 p.

DILL, L. M. MS, 1967. Behavioural ecology of chum salmon (*O. keta*) and coho salmon (*O. kisutch*) alevins in the gravel. M.Sc. Thesis. Univ. of British Columbia, Vanvouver, B.C. 83 p.

KOSKI, K. V. MS, 1966. The survival of coho salmon from egg deposition to emergence in three Oregon coastal streams. M.Sc. Thesis. Oregon State Univ., Corvallis, Oreg. 84 p.

LAGLER, K. F. 1956. Freshwater fishery biology. Dubuque, Iowa. 421 p.

MARR, D. H. A. 1965. Factors affecting the growth of salmon alevins and their survival and growth during the fry stage. Ass. River Authorities, Year Book 1965: 1–9.

MASON, J. C. 1969. Hypoxial stress prior to emergence and competition among coho salmon fry. J. Fish. Res. Bd. Canada 26: 63–91.

MCNEIL, W. J. 1963. Quality of the spawning bed as it relates to survival and growth of pink salmon embryos and alevins and time of fry emergence. Proc. 16th Int. Congr. Zool. 1: 242 (Abstr. only).

1966. Effect of the spawning bed environment on reproduction of pink and chum salmon. U.S. Fish Wildlife Serv. Fish. Bull. 65: 495–523.

1969. Survival of pink and chum salmon eggs and alevins, p. 101–117. *In* T. G. Northcote [ed]. Salmon and trout in streams. H. R. MacMillan Lectures in Fisheries, Univ. of British Columbia, Vancouver, B.C.

MCNEIL, W. J., AND W. H. AHNELL. 1964. Success of pink salmon spawning relative to size of spawning bed materials. U.S. Fish Wildlife Serv. Spec. Sci. Rep. Fish. 469: 1–15.

PHILLIPS, R. W. 1964. The influence of gravel size on survival to emergence of coho salmon and steelhead trout. Proc. N.W. Fish Cult. Conf., Oregon State Univ., Corvallis, Oreg. p. 90 (Abstr. only).

1965. Effect of fine material on salmon and trout redds, p. 59–64. *In* Proceedings of the meeting on erosion and sedimentation in the northwest, 1964–65 flood season. U.S. Dep. Agr. Soil Conserv. Serv. Portland, Oregon.

PHILLIPS, R. W., AND H. J. CAMPBELL. 1962. The embryonic survival of coho salmon and steelhead trout as influenced by some environmental conditions in gravel beds. Pac. Mar. Fish. Comm. Rep. 14: 60–73.

SANO, S. 1955. Observations on the natural spawning of the salmon, *Oncorhynchus keta*. Conditions of the spawning bed. Sci. Rep. Hokkaido Fish Hatchery 10: 1–6.

SHELTON, J. M. 1955. The hatching of chinook salmon eggs under simulated stream conditions. Progr. Fish-Cult. 17: 20–35.

SHUMWAY, D. L., C. E. WARREN, AND P. DOUDOROFF. 1964. The influence of oxygen concentrations and water movement on the growth of steelhead trout and coho salmon embryos. Trans. Amer. Fish. Soc. 93: 342–356.

STUART, T. A. 1953. Spawning migration, reproduction, and young stages of Loch trout (*Salmo trutta* L.). Scot. Home Dep. Freshwater Salmon Fish. Res. 5: 1–39.

Marine Borer Activity in Test Boards Operated in the Newfoundland Area during 1967–68[1,2]

JOHN W. EVANS

Marine Sciences Research Laboratory
Memorial University of Newfoundland, St. John's, Nfld.

EVANS, J. W. 1970. Marine borer activity in test boards operated in the Newfoundland area during 1967–68. J. Fish. Res. Bd. Canada 27: 201–203.

Marine wood borer activity is reported on the basis of an examination of 26 test panels that were submerged for approximately 1 year (from May 1967 to June 1968) at selected locations in the Newfoundland area. *Limnoria lignorum* (Rathke) was present consistently at each location, whereas *Teredo navalis* Linnaeus and *Psiloteredo megotara* (Hanley) were relatively uncommon.

Received August 29, 1969

DESTRUCTION OF WOOD by marine borers has been reported in the Newfoundland area by the W. F. Clapp Laboratories Inc. of Batelle Memorial Institute, Duxbury, Massachusetts, who have been operating special test boards here from time to time since 1936. Their observations on marine wood borer activity were restricted to Corner Brook and Lomond on the west coast and Argentia on the southeast coast of the island.

The present study reports the incidence and attack ratings of Teredinidae and Limnoridae during the 1967–68 season in locations selected to cover most of the coastal areas of Newfoundland.

---

[1]Contribution No. 39 from the Marine Sciences Research Laboratory, Memorial University of Newfoundland.

[2]Studies in Biology from Memorial University of Newfoundland, No. 167.

Printed in Canada (J1578)

Materials and methods — A total of 48 wooden test units were placed in selected harbours around Newfoundland in May–June 1967, of which 26 were recovered in June–July of the following year (Fig. 1).

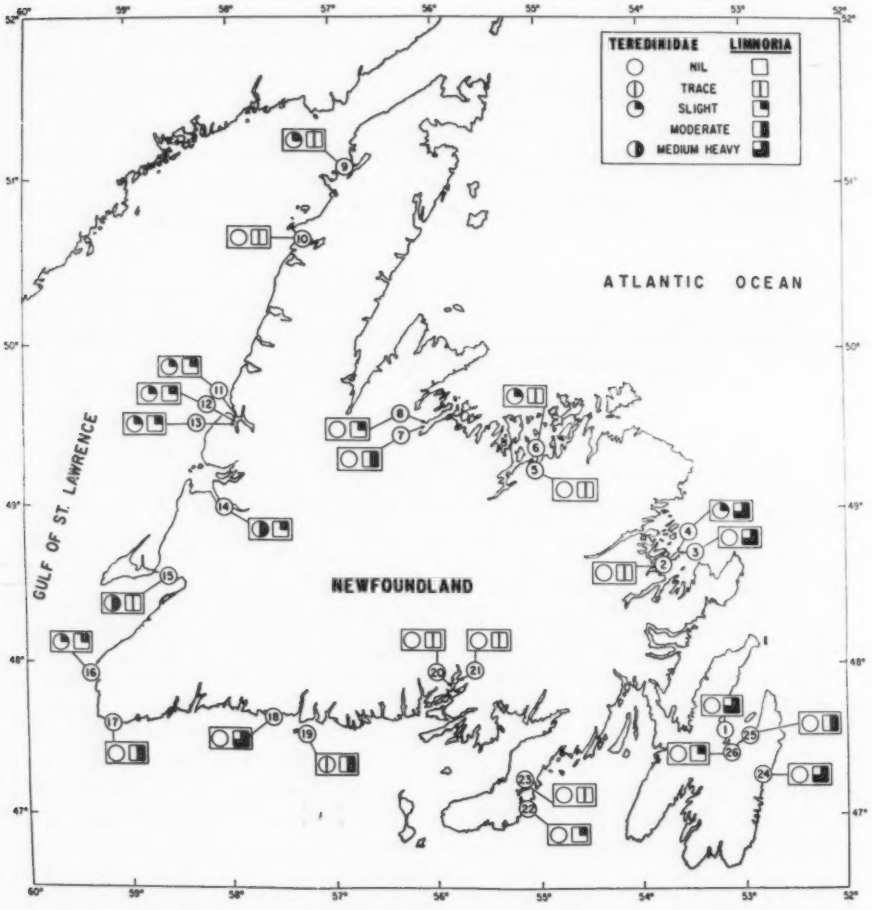

FIG. 1. Distribution of test blocks. Intensity of attack by Teredinidae and Limnoridae is shown.

A test unit consisted of a pair of 12 × 6 × 1-inch spruce boards, a 30-lb cement anchor, and a monofilament line for attaching the unit to wharf pilings. The boards hang about 2 ft above the bottom.

The test boards were examined for *Limnoria* by superficial examination and for *Teredo* by sawing off half-inch slices from the ends and by splitting the boards longitudinally. The criteria used in evaluating the severity of attack are those of the Clapp Laboratories Report (1950, p. 17–18). However, a 12-month, instead of an 8-month, period was used (Table 1).

TABLE 1. Criteria used in evaluating the degree of attack by Teredinidae and Limnoridae using standard tests boards (12 × 6 × 1 inch spruce), 12-month basis.

Teredinidae No. tunnels per panel	Limnoridae No. tunnels/sq inch	Attack rating
Up to 5	1	Trace
6–25	10	Slight
26–100	25	Moderate
101–250, or 50% filled	50	Medium heavy

Results — Three species of borer were observed: *Limnoria lignorum* (Rathke), *Teredo navalis* Linnaeus, and *Psiloteredo megotara* (Hanley). Figure 1 summarizes the attack ratings of these borers.

Teredinidae were only present in 10 of the 26 pairs of test boards examined, with the heaviest attack occurring on the west coast of the island and with scattered attacks in Bonavista and Notre Dame bays. Test units from the east and most of the south coasts failed to show any trace of Teredinidae. *Teredo navalis* was the most common shipworm at most infested stations except for the Bonne Bay area (block 11, 12, and 13) and Plum Point (block 9) where *Psiloteredo megotara* predominated. The specimens of *T. navalis* were mostly small (1.0–2.0 cm) whereas those of *P. megotara* were larger, attaining lengths up to 15 cm.

Limnoria occurred with uneven abundance at all stations.

Discussion — The study indicates that *Limnoria* is the most important and widespread borer of submerged timbers in the coastal waters of Newfoundland. The intensity of attack by this species varied but there was no obvious pattern to indicate that this variation was related to geographical location.

The Teredinidae, on the other hand, have a much more limited distribution, being found predominantly on the west coast and less abundantly in Notre Dame and Bonavista bays. The Clapp Laboratories Report (1951) mentions a very rare teredinid occurrence at Argentia. A number of old pilings (about 15 years old) from Heart's Content, Trinity Bay, were examined for evidence of shipworm attack. The most heavily infested contained only five Teredinidae burrows. These were large, to 1 cm in diameter and to 130 cm in length, and were probably the work of *Psiloteredo megotara*.

Acknowledgments — I would like to acknowledge the assistance of Dr Ruth Turner of the Museum of Comparative Zoology, Harvard University, who checked the identity of the Teredinidae.

This work was supported by a grant to the author from the National Museum of Canada. The specimens will be deposited at the Museum.

REFERENCES

CLAPP, W. F., LABORATORIES INC. 1950. Harbour reports on marine borer activity compiled for the Bureau of Yards and Docks, Department of Navy, Washington, D.C. 105 p.

1951. Fourth progress report on marine borer activity in test boards operated during 1950. Bur. Yards Docks, Dep. Navy, Washington, D.C., Rep. 7550. 80 p.

Graphical Estimation of Rates of Mortality and Growth

ROGER H. GREEN

Department of Zoology
University of Manitoba, Winnipeg 19, Man.

GREEN, R. H. 1970. Graphical estimation of rates of mortality and growth. J. Fish. Res. Bd. Canada 27: 204–208.

A method is presented by which the individual growth rate and the population mortality rate can be estimated graphically in certain populations, given only knowledge of maximum individual size and of average size at two times of the year. The four graphs that are given may be used in several different ways, depending on the information available.

Received September 16, 1969

THE ECOLOGIST often is interested in estimating the rate of growth in individual size and the mortality rate for a natural population. Accurate estimation of such parameters usually requires at least a year of intensive study and the expenditure of many man hours. For many purposes, such as an initial survey, a fairly rough idea of the growth rate and mortality rate would be of value.

The method presented here permits rapid graphical estimation of these two parameters, given only (*a*) time of recruitment of young-of-the-year; (*b*) average size of individuals in the mixed year-class population at two times of the year; (*c*) maximum size, or that size at which the growth rate approaches zero; and (*d*) several fairly restrictive assumptions (listed below in Derivation section) about the life history and the age-specific growth rates. The graphs may be used in several ways, depending on the information available.

Derivation — Let t = time when average size is estimated, in terms of $t = 0$ when recruitment of the new year-class is complete, and $t = 1$ just before recruitment a year later. Rates are therefore "per year." Therefore t = age of youngest year-class. Let l_t, l_{t+1}, etc. = average size of individuals in year-classes aged t, $t + 1$, etc.; $\bar{l}_t$ = average size of individuals in mixed year-class population at time of year t; l_∞ = asymptotic maximum size; and N_t, N_{t+1}, etc. = number surviving in year-class aged t, $t + 1$, etc.

Given that: (1) growth in size, however measured (length, volume, weight, etc.), can be approximately described by a function: $l_t = l_\infty(1 - e^{-Kt})$, with initial instantaneous growth rate K (von Bertalanffy, 1938;) (2) the instantaneous mortality rate Z is approximately constant over time, that is: $N_t = N_0 e^{-Zt}$; (3) recruitment from reproduction is concentrated in a single short span of time (say, 95% of recruitment within a 1 month span) each year, that is: $\sigma^2_{t=0}$ is small; and (4) the fraction of a cohort that attains an age of greater than 2 years is small (not greater than, say, 5%),

Printed in Canada (J1598)

then

$$\bar{l}_t = \frac{(N_t)\ (l_t) + (N_{t+1})\ (l_{t+1})}{N_t + N_{t+1}} \tag{1}$$

and

$$N_t = \frac{(N_{t+1})\ (l_{t+1}) - \bar{l}_t)}{\bar{l}_t - l_t}. \tag{2}$$

Since

$$N_{t+1} = N_t\ e^{-Z},$$

therefore

$$\bar{l}_t = \frac{l_t + l_{t+1}\ e^{-Z}}{1 + e^{-Z}}. \tag{3}$$

Since

$$l_t = l_\infty(1 - e^{-Kt})$$

and

$$l_{t+1} = l_\infty(1 \quad e^{-K(t+1)}),$$

therefore

$$\bar{l}_t/l_\infty = \frac{1 + e^{-Z} \quad e^{-Kt} \quad e^{-(K(t+1)+Z)}}{1 + e^{-Z}}. \tag{4}$$

With an estimate of l_∞ (largest animal observed at either time, or from independent information) and of average individual size at two times of the year, equation 4 yields two equations in two unknowns.

Application — Given values for all variables except Z and K in these two equations, Z and K are completely determined. They may be solved by trial and error, and if a computer program for solution of nonlinear simultaneous equations is used, the exact solution can be found rapidly. However, since it is doubtful that such exactness would be justified by the data, a generally useful procedure is to graph values of Z and K against axes scaled in values of $\bar{l}_t/l_\infty$ at the two times.

Figures 1–4 are four such graphs for four combinations of two times of the year. Estimation of K and Z is more precise when the first time is close to $t = 0$ (very soon after recruitment). This is because both mortality and growth, in terms of actual numbers of animals dying or units of size gained, asymptotically approach zero. Therefore their effects on year-class numbers and year-class size rapidly decrease with time.

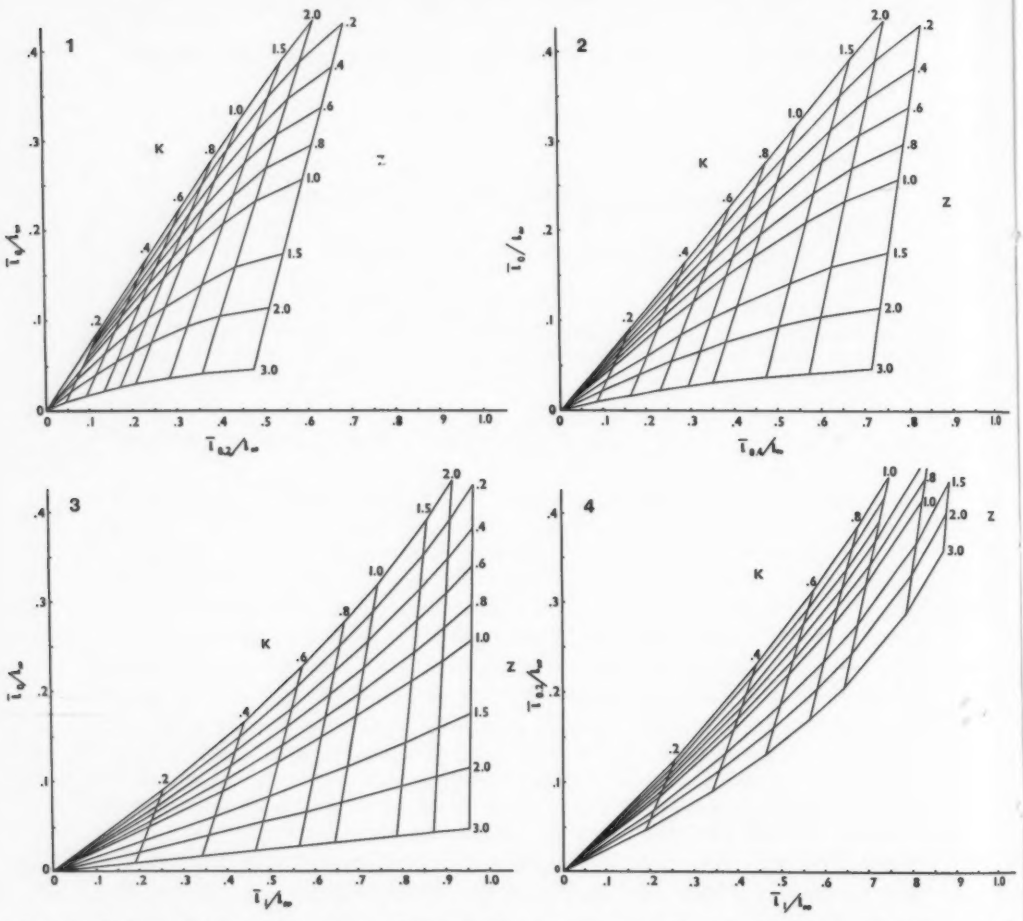

FIG. 1. Values of the growth parameter K and the instantaneous mortality rate Z (both per year) in terms of ratios of average size to maximum size for times $t = 0$ and $t = 0.2$ (see text).

FIG. 2. Legend as for Fig. 1, but for times $t = 0$ and $t = 0.4$.

FIG. 3. Legend as for Fig. 1, but for times $t = 0$ and $t = 1$.

FIG. 4. Legend as for Fig. 1, but for times $t = 0.2$ and $t = 1$.

Examples — (1) To illustrate the method, let us consider an imaginary species population with the following characteristics: $l_\infty = 0$, K = 0.5, and Z = 0.69 (e^{-Z} = 50% mortality per year). The average size of an individual in this population just after recruitment has ended ($t = 0$) is given by substituting the above into equation 4, yielding $\bar{l}_0/l_\infty = 0.131$; for a year later, just before the next recruitment, substitution of $t = 1$ into equation 4 yields $\bar{l}_1/l_\infty = 0.473$.

If these were ratios of observed average sizes to maximum size just after and just before reproduction, then we would go to Fig. 3 and locate the point defined by $\bar{l}_0/l_\infty = 0.131$ and $\bar{l}_1/l_\infty = 0.473$, which indicates that approximate values for K and Z are, respectively, 0.5 and 0.7.

(2) For *Gammarus lacustris* in West Blue Lake, Manitoba, and probably for many other cold-temperate aquatic species as well, growth virtually ceases in winter. Recruitment through reproduction occurs in June, so that the above growth model cannot be assumed to apply for an entire year. However, it could reasonably apply during the growing season, estimated in this instance to be approximately 6 months. (There is of course an assumption here that neither growth nor mortality are significant during the winter months.)

West Blue Lake *G. lacustris* on June 26 had an average length of 5.4 mm and on November 25 (approximately 0.4 of a year later) an average length of 9.8 mm. The largest observed *G. lacustris* were 17 mm in length, so 17 mm was used as an estimate of l_∞. Therefore $\bar{l}_0/l_\infty = 5.4/17 = 0.3$ and $\bar{l}_{0.4}/l_\infty = 9.8/17 = 0.6$. If we go to Fig. 2 we find that these values yield estimates of $Z = 0.4$ per year and $K = 1.4$ per year. The "year" to which the model applies is a 6-month growing year, and independent estimates of Z and K during this period are 0.4 per 6 months and 2.1 per 6 months, respectively.

Discussion — Although I feel that this procedure is potentially useful, I should emphasize that there is also potential for misuse. It is not intended that growth and mortality rates should be estimated by this method without subsequent verification by completely independent methods. On the other hand, in many instances the best procedure for precisely estimating these parameters is determined in part by their approximate magnitude, and this method may be used as an aid to this first approximation.

If l_∞ is not known information may still be obtained about K or Z, or both. (Of course, if either K or Z can be estimated independently, then the other parameter can be determined more precisely than if neither were known.) For example, if the average size at the first time is one-fifth of that at the second time, then the correct values for Z and K fall somewhere along a line passing through the origin with a slope of one-fifth on the appropriate graph. If $\bar{l}_0 = 0.2$ ($\bar{l}_1$), then $Z = 1.0$–1.5 over a wide range of K.

If average individual size is known for three times of the year, then one has enough information for three equations in three unknowns (K, Z, and l_∞). These could be solved by trial and error, or on a computer; alternatively, if l_∞ can be estimated, then the two intervals formed by the three times can be used as above, providing two estimates of K and Z.

If growth in size would be expected to follow some pattern other than the given one for some species population, then equation 4 could be modified accordingly and appropriate graphs constructed. For example, if growth were linear throughout all or a large part of the 2-year period, then equation 4 would become:

$$\bar{l}_t = \frac{K(t(1 + e^{-Z}) + e^{-Z})}{1 + e^{-Z}} = K\left[t + \frac{e^{-Z}}{1 + e^{-Z}}\right].$$

The parameter l_∞ does not, of course, appear in these equations and only average sizes at two times are needed to estimate K and Z. In this instance K is not an instantaneous rate but actual growth per year in the units of size used. Any growth function containing one unknown parameter could be used.

Acknowledgments — I would like to thank Dr A. L. Hamilton for critically reviewing this paper in the manuscript stage, and also Mr Ray Biette for the use of data on *Gammarus lacustris* from his M.Sc. thesis.

REFERENCES

BERTALANFFY, L. VON 1938. A quantitative theory of organic growth. Human Biol. 10: 181–213.

Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles have been included in an annual volume of "Studies," composed of two parts. The final binding is the 1969 Studies, Part 2, which included No. 1355. These will no longer be bound and distributed. We will continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The boldface number following each title refers to the FRB establishment listed below.

(**1**) Fisheries Research Board of Canada
Biological Station
P.O. Drawer 100
Nanaimo, British Columbia

(**2**) Fisheries Research Board of Canada
Research Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(**3**) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg 19, Manitoba

(**4**) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Québec

(**5**) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(**6**) Fisheries Research Board of Canada
Atlantic Oceanographic Group
Bedford Institute of Oceanography
P.O. Box 1006
Dartmouth, Nova Scotia

(**7**) Fisheries Research Board of Canada
Research Laboratory
P.O. Box 429
Halifax, Nova Scotia

(**8**) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

Studies No.

1356. Fletcher, G. L., D. C. Hardy, and D. R. Idler. 1969. Testosterone production and metabolic clearance rates in sexually mature male and female skate (*Raja radiata*). Endocrinology 85: 552–560. (**7**)

1357. Idler, D. R., and H. C. Freeman. 1969. Sex hormone binding proteins I. Binding of steroids by serum of an elasmobranch (*Raja radiata*). Gen. Comp. Endocrinol. 13: 75–82. (**7**)

1358. Kabata, Z. 1969. Copepoda parasitic on Australian fishes IX. Family Chondracanthidae. J. Natur. Hist. 3: 497–507. (**1**)

1359. Whyte, J. N. C. 1969. Proton magnetic resonance and mass spectrometric studies on derivatives of sodium 2-*O*-α-D-mannopyranosyl-D-glycerate and related compounds. Can. J. Chem. 47: 4083–4086. (**2**)

1360. Holmes, W. N., and E. M. Donaldson. 1969. The body components compartments and the distribution of electrolytes. Fish. Physiol. 1: 1–89. (**2**)

1361. Muir, B. S. 1969. Further observations on gill modifications of oceanic fishes. Copeia 1969: 629. (**6**)

1362. Freeman, H. C., and D. R. Idler. 1969. Sex hormone binding proteins II. Isolation from serum of an elasmobranch (*Raja radiata*). Gen. Comp. Endocrinol. 13: 83–91. (**7**)

1363. MacCallum, W. A., J. I. Jaffray, D. A. Chalker, and D. R. Idler. 1969. Condition of Newfoundland offshore fish and its influence on quality after single and double freezing, p. 56–63. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Limited, London, England. (**7**)

1364. MacCallum, W. A., and D. R. Idler. 1969. Influence of thawing and thawing methods on the immediate and refrozen storage quality of fish, p. 213–223. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Limited, London, England. (7)

1365. Dyer, W. J., and J. Peters. 1969. Factors influencing quality changes during frozen storage and distribution of frozen products, including glazing, coating and packaging, p. 317–322. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Limited, London, England. (7)

1366. Dyer, W. J. 1969. Effect of brining and polyphosphate on yield and quality, p. 167–171. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Limited, London, England. (7)

1367. Power, H. E., M. L. Morton, and R. E. Sinclair. 1969. The chemical and physical characteristics of cod stored at superchill temperatures, p. 104–113. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Limited, London, England. (7)

1368. MacCallum, W. A., and K. Sĕpić. 1969. Commercial aspects of reprocessing and marketing of sea frozen fish, p. 240–251. *In* R. Kreuzer [ed.] Freezing and irradiation of fish. Fishing News (Books) Limited, London, England. (7)

1369. Seki, H., and J. Fulton. 1969. Infection of marine copepods by *Metschnikowia* sp. Mycopathol. Mycol. Appl. 38: 61–70. (1)

1370. Odense, P. H., T. C. Leung, T. M. Allen, and E. Parker. 1969. Multiple forms of lactate dehydrogenase in the cod, *Gadus morhua* L. Biochem. Genet. 3: 317–334. (7)

1371. Brockerhoff, H. 1969. Esters of phenols as substrates for pancreatic lipase. Biochim. Biophys. Acta 191: 181–183. (7)

1372. Idler, D. R., M. J. O'Halloran, and D. A. Horne. 1969. Interrenalectomy and hypophysectomy in relation to liver glycogen levels in the skate (*Raja erinacea*). Gen. Comp. Endocrinol. 13: 303–306. (7)

1373. Fagerlund, U. H. M. 1969. Lipid metabolism, p. 123–134. *In* Chemical zoology, Vol. III. Academic Press, Inc., New York. (2)

1374. Brockerhoff, H. 1969. Action of pancreatic lipase on emulsions of water-soluble esters. Arch. Biochem. Biophys. 134: 366–371. (7)

1375. Ricker, W. E., and H. H. Ross. 1969. The genus *Zealeuctra* and its position in the family Leuctridae (Plecoptera, Insecta). Can. J. Zool. 47: 1113–1127. (1)

1376. Licht, P., and E. M. Donaldson. 1969. Gonadotropic activity of salmon pituitary extract in the male lizard (*Anolis carolinensis*). Biol. Reprod. 1: 307–314. (2)

1377. Power, H. E., K. A. Savagaon, B. E. March, and J. Biely. 1969. Assessment of the nutrient content of Atlantic coast herring fish meals. Feedstuffs 41: 48–49. (7)

1378. Muir, B. S., and G. M. Hughes. 1969. Gill dimensions for three species of tunny. J. Exp. Biol. 51: 271–285. (6)

1379. Mercer, M. C. 1969. Biological characteristics of migrant ommastrephid squid, *Illex illecebrosus* (LeSueur), in the Newfoundland area. Amer. Zool. 9: 505. (8)

1380. Sandeman, E. J. 1969. Diurnal variation in availability of different sizes of redfish, *Sebastes mentella*. Int. Comm. Northwest Atl. Fish. Res. Bull. 6: 35–46. (8)

1381. TEMPLEMAN, W. 1969. Experimental snella fishing for cod, *Gadus morhua* L., on West Greenland Bank, August 1965, and comparison of a snella and an otter-trawl catch of cod from the same locality of Store Hellefiske Bank. Int. Comm. Northwest Atl. Fish. Res. Bull. 6: 53–64. (8)

1382. SANDEMAN, E. J. 1969. Age determination and growth rate of redfish, *Sebastes* sp., from selected areas around Newfoundland. Int. Comm. Northwest Atl. Fish. Res. Bull. 6: 79–106. (8)

1383. LEAR, W. H. 1969. Length–weight relationship of commercial-size Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum). Int. Comm. Northwest Atl. Fish. Res. Bull. 6: 119–121. (8)

1384. TEMPLEMAN, W. 1969. The scientific name, distribution and characteristics of the blue ling, *Molva dypterygia* (Pennant), from West Greenland and Newfoundland areas. Fiskeridir. Skr. Ser. Havunders. 15: 145–162. (8)

1385. ACKMAN, R. G., S. N. HOOPER, M. KATES, A. K. SEN GUPTA, G. EGLINTON, AND I. MACLEAN. 1969. Phytanic acid L-menthyl esters gas–liquid chromatographic separation of diastereoisomers. J. Chromatogr. 44: 256–261. (7)

Guide to Authors

Details on typing and other items in preparing copy are given in a guide for typists. Also, a list of recommended spellings of about 100 words common in fisheries journals is available from the Office of the Editor.

EDITORIAL PRACTICES

An abstract must be provided in the same language as the text; authors are encouraged to provide both English and French versions.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. N.W., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopedique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

Illustrations

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

CHECKLIST ON MANUSCRIPTS

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

General

Is there new knowledge in the report worth publishing?

Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?

If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?

Are all the tables and figures needed, and organized for economy of space?

Would any of the text be clearer if condensed?

Should some of the data be made available separately, in a manuscript report or at a data repository?

Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

Guide for Typists Preparing Copy for

Journal of the Fisheries Research Board of Canada

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

Tables Examine the current volume of the *Journal* to see how tables are set up (Appendix C). Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, they are typed with the text, they seldom need horizontal lines, and the data are arranged in as few rows as possible (Appendix C).

Illustrations Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for Illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical expressions, equations, and formulas Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

References Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

Spellings A list of about 100 words common in fisheries journals is given in Appendix F.

Appendices Appendices A to F are available on request from the Office of the Editor.

Now Available:

Annual Report 1968 (Bilingual)

Fisheries Research Board of Canada

The Annual Report of the Fisheries Research Board of Canada contains lists of Board members, committee members, and officers; establishments and directors; the Chairman's review; research programs in 1968; research highlights; and expenditures by areas and disciplines.

Review 1967–1968

Fisheries Research Board of Canada

The biennial Review of the Fisheries Research Board of Canada contains lists of Board members, organization, establishments, reviews of investigations, professional staff by establishments, and lists of 1967 and 1968 publications and reports.

Copies of both the *Annual Report* and the *Review* are available free of charge from

Fisheries Research Board of Canada
Office of the Editor
116 Lisgar Street
Ottawa 5, Ontario

JUN 1 0 1970

Crown Copyrights reserved
Available by mail from Queen's Printer, Ottawa, Canada
and at the following Canadian Government bookshops:

OTTAWA
Daly Building, Corner Mackenzie and Rideau

TORONTO
221 Yonge Street

WINNIPEG
Mall Center Building, 499 Portage Avenue

VANCOUVER
657 Granville Street

or through your bookseller

Price $1.00
Catalogue No. Fs 1-1/1969-1

Price subject to change without notice

Queen's Printer for Canada, Ottawa, 1969